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Editor's Note

The increasing number of research workers in universities and colleges and other organizations—national and international—and the resulting increase in the number of scientific papers would perhaps justify the birth of the 'ENTOMON', a journal embracing all aspects of entomological research–pure and applied. It is in the fitness of things that the release of the first issue of the 'ENTOMON' should coincide with the first death anniversary of the renowned neuroendocrinologist, Prof. K. K. Nayar, whose contribution to various aspects of entomological research needs no elaboration. As chief editor, I have pleasure not only in welcoming scientific papers which would merit national as well as international recognition, but also constructive suggestions from entomologists, which would promote the cause of the journal, enabling it to maintain the highest standards.

T. N. ANANTHAKRISHNAN

Dedicated to

Professor K. K. NAYAR

Entomon, 1976, *Vol.* 1, *No.* 1, *pp.* 1 *to* 6.

K. K. NAYAR

1920 – 1975

Professor K. K. NAYAR, who was Head of the Department of Zoology in the University of Kerala, died in harness on 26th June 1975 at the age of fifty five, after a brief illness. To the wide circle of his students, colleagues, friends and well wishers all over the world the news came as a rude shock. Although he was ailing for the last few months of his life he was attending to all his duties regularly, and his sudden demise took all his associates by surprise.

Professor NAYAR was born on June 12th, 1920 at Kottarakara, Travancore state which is now part of Kerala State. He took his B.Sc. (Hons.) and M. A. in Zoology from the University of Madras in 1941 and 1947 respectively, and his Ph.D. from the erstwhile University of Travancore, now University of Kerala. He was Lecturer in Zoology in the University of Travancore from 1945 to 1957, and with the taking over of the University College by the State Government, he worked as Professor in Government Victoria College, Palghat, Government College, Chittoor and University College, Trivandrum, all under the Collegiate Education Service of the Government of Kerala. During this period he had perhaps the most frustrating experience in his research career as very often, he and his research team had to work in institutions where even the students' microscope was a luxury. However, the urge and motivation for research burned in him incessantly and it kindled fire in those around him and led them along their chosen path of research. That stormy period of trials and tribulations came to a close in 1963 when he became Reader and Head of the newly formed Department of Zoology in the University of Kerala in 1963 at Calicut Centre. Subsequently he became Professor in the same Department. Meanwhile international recognition and funds were forthcoming for his various research projects both from the Ford Foundation and PL 480. A new Research Unit for Reproductive Biology was established at Trivandrum, to which he shifted along with his research team, but he continued as Professor and Head of the Department of Zoology at Calicut Centre as well. A separate university, the University of Calicut, was established at Calicut Centre and Professor NAYAR was appointed Head of the Department of Zoology in the University of Kerala at Trivandrum in 1968. Subsequently the Department of Zoology as well as the Reproductive Biology Research Unit shifted to the present Kariavattom Campus in the subrubs of Trivandrum city.

Professor NAYAR started his research work on gall midges. His Ph.D. thesis dealt with biology and systematics of gall midges. He had conducted a survey of these minute insects of this region, discovered some new species of gall midges and elucidated new concepts on the biology of many of these insects. Eventhough he subsequently shifted his field of research, gall midges continued to interest him till his death.

As a result of his early contacts with research workers in Copenhagen and England, especially with Professor V. B. WIGGLESWORTH who had profound influence on him and whom he almost adored, Insect Endocrinology and Neurosecretion became his

main fields of research. This interest subsequently widened and he became an active worker in the field of Comparative Endocrinology. He was undoubtedly the foremost authority in Comparative Invertebrate Endocrinology in India and was well known internationally for his work in this field. He chose the insect *Iiphita limbata* for most of his early endocrinological work and during the fifties and the early sixties made many significant contributions to the understanding of reproduction in the female of this species including oviposition and water balance. During this period he also worked on the endocrine mechanism of certain Diptera and Lepidoptera including control of metamorphosis in these animals. As a result of his outstanding contribution during this period, the Ford Foundation offered him a research grant with which he equipped the laboratory with sophisticated research instruments, including an electron microscope. It also enabled him to establish a good departmental library and to train students in the field of Reproductive Biology of Insects. This financial help was further supplemented by PL 480 grant for the study of crustacean biology and smaller grants from the Atomic Energy Department of India for the study of the effects of natural radiation of the sandy shore of Kerala on its soil microfauna, from the Tea Board of India for the study of micro-organisms inhabitating the soil of tea gardens and an ICAR grant for the study of Frog Biology. Though his chief interest was Comparative Endocrinology of invertebrates, he also supervised research projects in endocrinology of other animals including various groups of vertebrates, and in such unrelated fields as Comparative Oncology, Regeneration and Wound Healing, Pheromones, Ethology, Invertebrate Reproductive Biology, Chromosome Cytology, Soil Biology, Radiation Biology and in Systematics. In these fields he has a number of publications and has successfully guided seven research students. At the time of his tragic death he was guiding six research students in such diverse fields as Pheromones in Social Insects, Invertebrate Immunity, Morphogenetic action of insect hormones, Differentiation of cuticular pattern in insects, Neurosecretion in the shrew and Comparative aspects of endocrine pancreas in lower vertebrates. This is really surprising in this age of narrow specialisation. As a recognition of his contributions in these fields he was elected Fellow of the Indian National Science Academy in 1971 and of the Indian Academy of Sciences in 1974.

Professor NAYAR has travelled widely and was a familiar figure at a number of National and International symposia and conferences: he has also chaired many of the sessions and has presented papers at these scientific gatherings. The Third International Symposium on Neurosecretion held n Bristol in the year 1961, International Symposium on Invertebrate Endocrinology in Jena in 1965, International Conference on Insect Endocrines held in Brno, Czechoslovakia in 1966, International Conference on Comparative Endocrinology held in Delhi in 1967, International Symposium on Neoplasm and related disorders of Invertebrates and lower vertebrates held in Washington D. C. in 1968 and the 5th International Congress of Endocrinology held in Mexico city in 1968, were some of the conferences in which he actively participated.

During 1953–1954, Professor NAYAR was also a Guest Research Worker in the Rothamstead Experimental Station, Harpenden, Herts, England; a trainee in Neurosecretion in the Royal Veterinary College, Copenhagen and in the University of Oxford. In these institutions he worked on insect endocrines. He was elected Commonwealth Visiting Professor and worked in the School of Biological Sciences in the University of

East Anglia, Norwich, England in 1968–1969 on Comparative Oncology. He was Guest Professor in the Department of Anatomy of the Albert Einstein College of Medicine, Bronx, New York and in the Department of Zoology in the University of California, Berkeley, in 1969 for short periods. As Ford Foundation grantee he visited various endocrinological laboratories in U. S. A. and Japan in 1964. All these opportunities brought him in close contact with such top-ranking scientists abroad as Professors H. A. BERN, E. SCHARRER, B. SCHARRER, B. HANSTROM, V. B. WIGGLESWORTH and a number of others.

Professor NAYAR has also written a number of books. In his "Elements of Insect Endocrinology" (Prentice Hall, India) published in 1973, which was based on a series of lectures given by him in various Indian Universities under the U. G. C. Lecture programme, he has brought the rapidly advancing frontiers in this field within easy reach of general zoologists and it has been a boon to graduate and post graduate students in India. The release of the book "Principles of General and Applied Entomology" (co-authors: Dr. T. N. ANANTHAKRISHNAN and Dr. B.V. David, Tata McGraw Hill) has been announced while the manuscript of his work on "Invertebrate Reproduction" is in press.

Professor NAYAR was not only an eminent scientist, but a teacher and academician *par excellence*. The depth of his knowledge in various branches of Zoology was unfathomable. Zoologists in general found a discussion with him extremely stimulating and rewarding. He conveyed the most complicated ideas in extremely lucid and simple manner with heart and soul in what he said and the result was a lively class most sought after. He was very much concerned about the falling standards in Indian Universities and fought to bring curriculum in Indian Universities in tune with latest developments in science. He has organised a number of Summer Institutes at national level which brought participants from many teaching institutions in contact with leading scientists in India and abroad, and stimulated interest in research and in modern trends in Zoology, among participants. He was associated with a number of state and national committees of Science, Technology and Education and with various academic bodies and activities of many Indian Universities. He was a member of many scientific societies and the Vice-President of the Ethological Society of India. He was also on the editorial board of Journal of Animal Morphology and Physiology. During the last days of his life Professor NAYAR felt the growing need for a scientific journal to cover various aspects of Entomology. Towards this goal he called a meeting on 28th April 1975. At this meeting it was decided to form the Association for Advancement of Entomology to bring together scientists working on diverse aspects of this field and steps were initiated to publish ENTOMON to be the official organ of the Association. Professor NAYAR was unanimously elected the Editor-in-Chief of the journal, and was leading the Association and the journal when death overtook him.

Despite all his brilliant academic achievements he was unassuming and extremely kind, and encouraged other scientists, especially those of the younger generation who struggled hard for their survival. He respected the views of even the beginners just as well as those of eminent scientists. He was also an idealist who believed that scientific work should be pursued for the elucidation of truth only. He not only professed it but had the courage and tenacity to practise what he preached, even in the face of stiff adversity. He was rather quiet by nature,

but when he talked, he did it most effectively and in few words. His conversation was often full of humour. In his untimely death, India and the world lost a great Zoologist. He leaves behind him his aged mother, his wife, daughter and three sons.

Acknowledgements:— This article is compiled from the personal files of Professor K. K. NAYAR. I thank Prof. NAYAR's family for making available to me these papers for reference, and to Prof. K. M. ALEXANDER of this Department for co-operation in this matter and for various suggestions.

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IN VITRO ANALYSIS OF THE INSECT NEURO-ENDOCRINE ORGANS

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Cultures of neurosecretory cells and corpus cardiacum of the cockroach *Periplaneta americana* display vigorous outgrowth of nerve fiber processes *in vitro*. Numerous cells of various types migrated out of the corpus cardiacum into the medium. Light and electron microscopic studies showed the presence of neurosecretory granules in the cell bodies and in the axonal processes. Neurosecretory cells were electrically active *in vitro* and the unit electrical activity of NS cells contrasted sharply with the bursting electrical discharges of the CC cultures. The synthesis and release of radiolabeled juvenile hormone by adult and nymphal corpora allata indicated that these glands are functionally active *in vitro*.

INTRODUCTION

The insect neuro-endocrine organs (the neurosecretory cells of the brain, the corpora cardiaca, the corpora allata and the ecdysial glands) have been extensively investigated ever since they were first discovered (reviewed by BERN & HAGADORN, 1965; DOANE, 1973; MADDRELL, 1974; WIGGLESWORTH, 1964). Only recently it became possible to study them with *in vitro* techniques because the tissue culture media used for vertebrate tissues proved to be unsuitable for insect tissues. The considerable technical difficulties encountered in dissecting out the small insect endocrine glands and in devising culture media suitable to maintain these organs *in vitro* have hampered progress in this field. We owe a great deal to the efforts made by T.D.C. GRACE, G. R. WYATT, S. S. WYATT and others (see C. VAGO, 1971; 1972) who devised culture media and techniques suitable for growing insect cells and tissues *in vitro*. With further refinements of their methods, we are now in a position to investigate whole organs, or individual tissues or cells of a variety of insects and other invertebrates *in vitro*. A chemically defined synthetic medium and tissue culture techniques were developed (CHEN & LEVI-MONTALCINI, 1969) which allowed *in vitro* studies on the embryonic nervous system of the cockroach (LEVI-MONTALCINI & CHEN, 1969). These studies were extended to the neuro-endocrine organs of nymphal and adult specimens (LEVI-MONTALCINI, 1971; LEVI-MONTALCINI & SESHAN, 1973). Fully differentiated cockroach neurosecretory cells, the corpora cardiaca and the corpora allata of nymphal and adult cockroaches were maintained in organ culture for several weeks during which period they exhibited neuronal fiber processes, cell migration and other structural and functional features (SESHAN & LEVI-MONTALCINI, 1971; SESHAN *et al.*, 1974). It is the object of this article to report in a condensed form the results of these and related investigations.

MATERIALS AND METHODS

Preparation of cultures

Nymphs and adult cockroaches (*Periplaneta americana*) of both sexes were used as donors for the neuro-endocrine organs. Brain, thoracic ganglia,

foregut and heart of 15 16 day old embryos and ovaries of nymphs and adults were dissected out and cultured together with the neuro-endocrine organs. The neurosecretory cells of the pars intercerebralis, the corpora cardiaca, the corpora allata of nymphs and adults, embryonic organs and ovarian follicles were dissected out and prepared as described elsewhere (SESHAN, 1976; SESHAN & LEVI-MONTALCINI, 1971). To minimize bacterial and fungal contamination of cultures, the tissue culture room was equipped with germicidal lamps and all dissections were performed in a sterile atmosphere. Three commercially available media were used: SCHNEIDER's *Drosophila* medium, GRACE's insect medium (Grant Island Biological Co.) and EAGLE's basal medium (Microbiological Associates). At the moment of use, SCHNEIDER's and EAGLE's media were mixed in the ratio 5:4 respectively. GRACE's medium custom made without the amino acid methionine was used exclusively for biosynthetic studies on the corpus allatum. This medium was supplemented with 1% bovine albumin (Fraction V from bovine plasma, Metrix, Division of Armour Pharmaceutical Co.). 2 microcuries/ml of (S-methyl-^{14}C) methionine (specific activities in the range of 47 to 58 Ci/mole (New England Nuclear) added to this medium served as a precursor for the juvenile hormone produced by the corpora allata. Other precursors used were 1 mg/ml each of mevalonolactone (Sigma Chemical Co.) and homomevalono-lactone. The latter was synthesized in our laboratory. Every fourth day the incubation media were collected and fresh media added to the cultures. The collected media were extracted and the hormone purified and identified by thin layer and high pressure liquid chromatography (DAHM *et al.*, 1976).

Histological and other techniques

i. GOMORI's chrome hematoxylin phloxin (GOMORI, 1941); aldehyde fuchsin and victoria blue (DOGRA & TANDON, 1964) to demonstrate neurosecretory activity in histological preparations. ii. CAJAL-DECASTRO silver staining technique for the demonstration of nerve fibers (LEVI-MONTALCINI & CHEN, 1969). iii. Inverted and differential interference contrast microscopy to study and photograph living cultures. iv. Electron microscopy for ultrastructural studies (SESHAN & LEVI-MONTALCINI, 1971). v. Electrophysiology to detect bioelectrical phenomena in living cultures (SESHAN *et al.*, 1974).

RESULTS

Neurosecretory (NS) cells, corpus cardiacum (CC) and corpus allatum (CA) were cultured in the presence of embryonic organs (brain, thoracic ganglia, foregut and heart) or nymphal and adult ovarian follicles. These organs were positioned at a distance of approximately 1–2 mm from the neuroendocrine organs. Previous studies had shown that these tissues increase the amount of nerve fiber outgrowth and survival of the embryonic nervous system (CHEN & LEVI-MONTALCINI, 1969; 1970; LEVI-MONTALCINI & CHEN, 1969). Our observations agree with these findings; in the absence of the above-mentioned organs, the neuro-endocrine explants show degenerative changes.

In vitro analysis of neurosecretory cells

Toward the end of the first week of incubation *in vitro*, nerve fiber processes emerge from one or more sectors of the NS explant which usually consisted of a large aggregate, or a small cluster or individual cells. During the second and third week, the outgrowing fibers lengthen further, assume a tubular appearance and give origin to many side branches (Fig. 1). When several NS explants are cultured together, in the same vessel, adjoining NS groups or individual cells establish contact to one another with nerve fiber processes. Meanwhile, many of the smaller nerve fibers originating from large NS clusters may coalesce to form axonal bundles and merge with similar fiber bundles originating from embryonic explants. With the establishment of neuro-fibrillar contact between NS and embryonic explants, e.g., foregut and heart, the latter organs begin to exhibit contractility. Whether the motility of the foregut and heart is of myogenic or neurogenic origin was not ascertained in these studies.

NS granules were evident in many cultured NS cells after staining with GOMORI's and other techniques. Axonal processes contained only few such granules. Ultrastructural studies showed numerous electron-

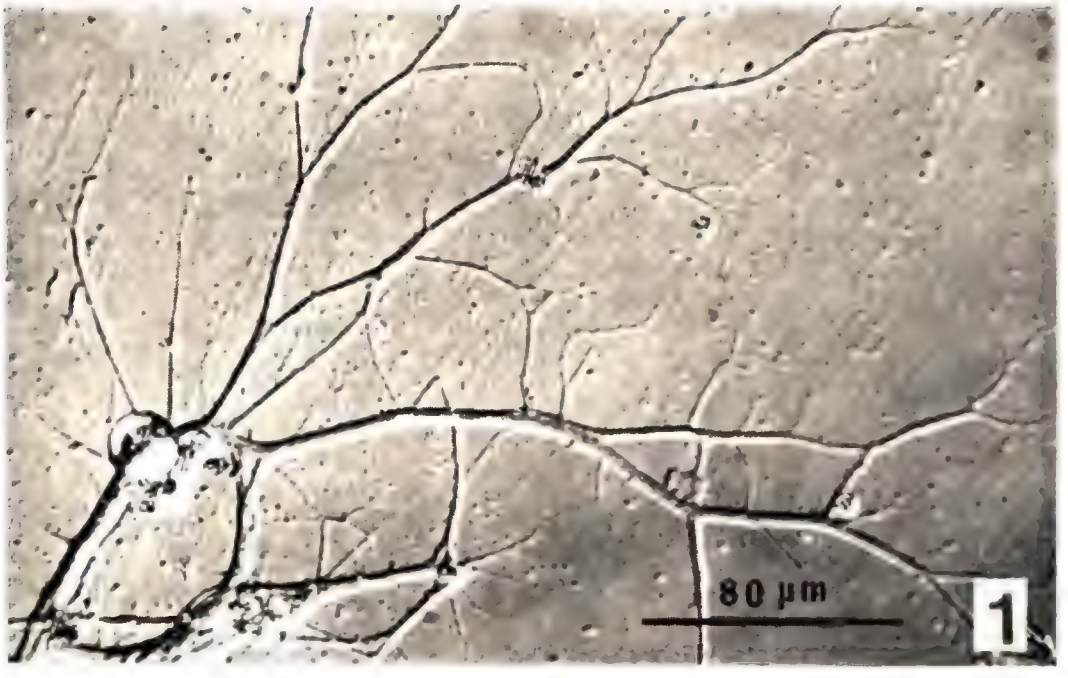


FIG. 1. Large axons and collateral fibers branch out from a small cluster of NS cell of a 7th instar nymph cultured *in vitro* for 33 days. One NS cell body is shown in this microphoto. Other NS cells (not shown) are in the adjacent frame.

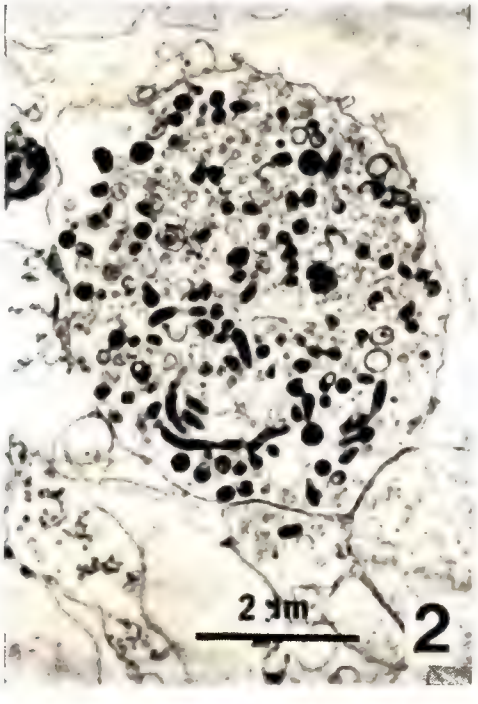


FIG. 2. An electron micrograph of axons growing out of an NS cluster of a 6th instar nymph maintained *in vitro* for 4 weeks. Note the numerous vesicles in the axon filled with varying gradations of electron dense material.

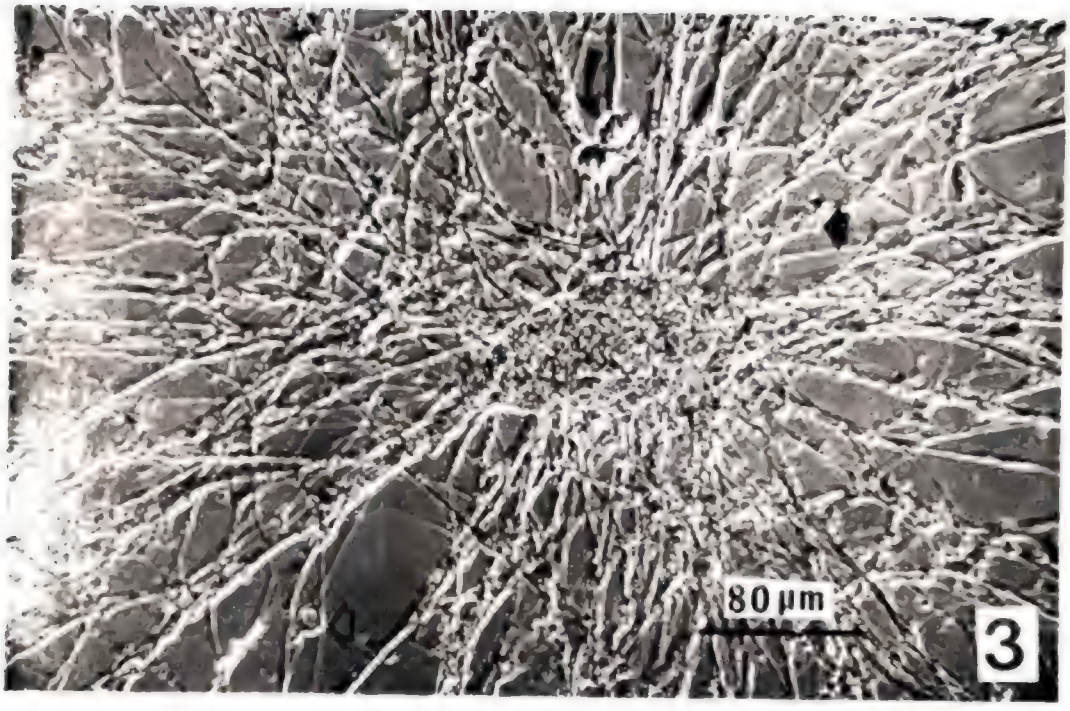
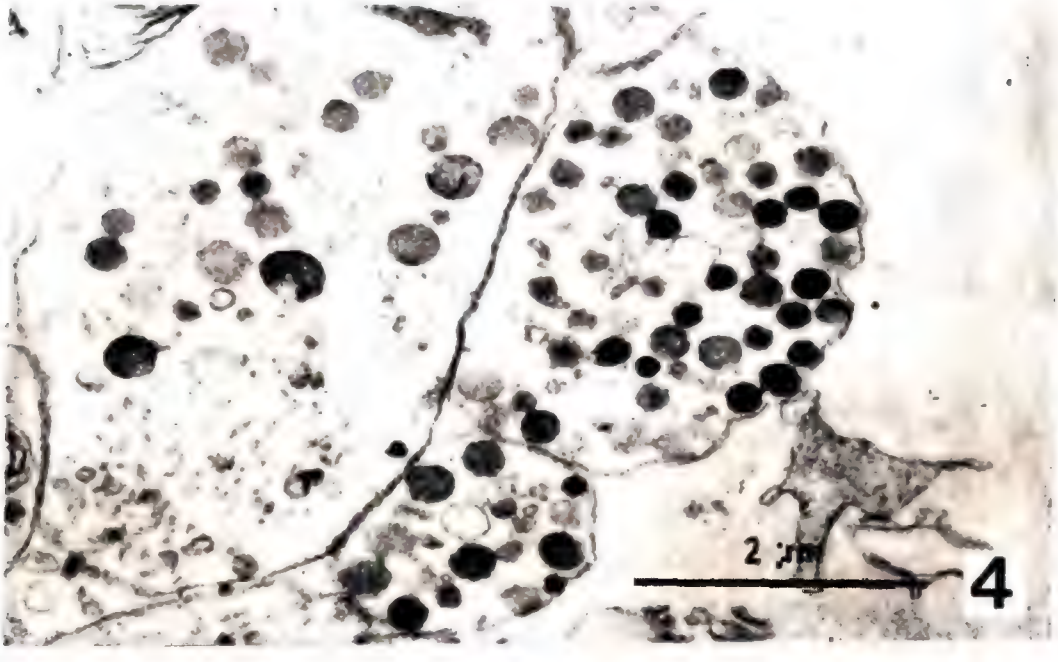


FIG. 3. Nomarski microphoto of a living culture of a segment of corpus cardiacum taken from a male adult and cultured *in vitro* for 47 days. Note the prolific outgrowth of axons, small glia-like cells (small closed arrows), large migrated neurons (long arrows), and a tracheolar cell (small open arrow).

FIG. 4. Electron micrograph of axons growing out of a CC segment of a male adult cultured *in vitro* for 47 days. Numerous electron dense granules fill the axons.



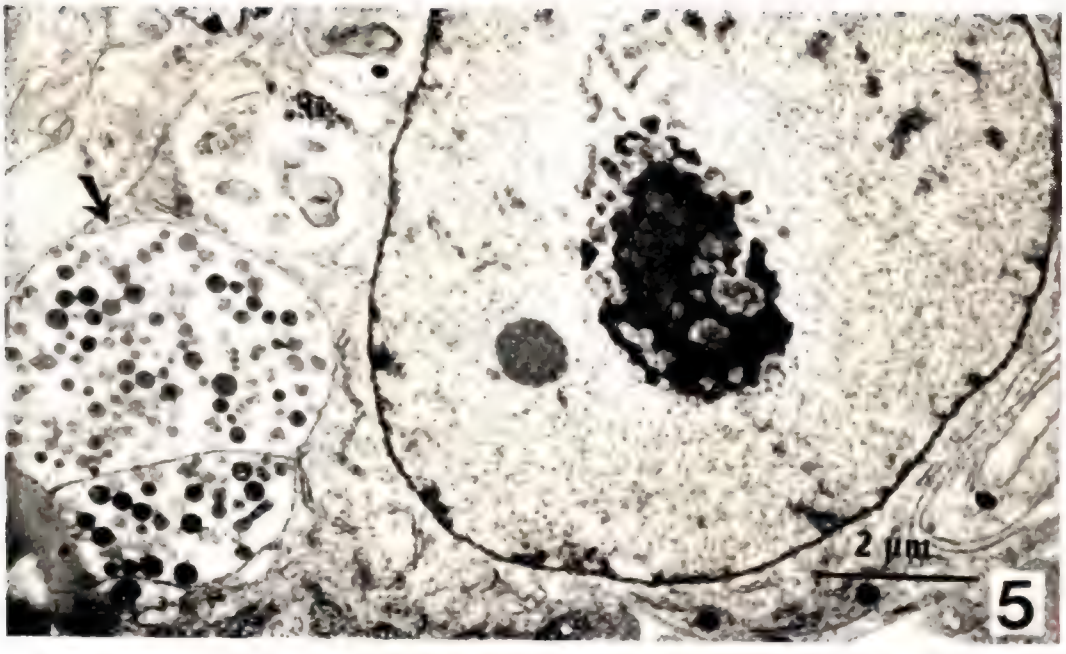
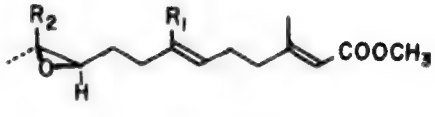


FIG. 5. Electron micrograph of cells of the corpus allatum of a 10th instar nymph maintained *in vitro* for 22 days. Cellular details and NS axons (arrow) are evident.



1	$R_1 = R_2 = -C_2H_5$	JH-I
2	$R_1 = -CH_3$, $R_2 = -C_2H_5$	JH-II
3	$R_1 = R_2 = -CH_3$	JH-III

FIG. 6. Structures of the known uvenile hormones.

dense NS granules in the cell pericarya and axons. In Fig. 2 profile of a large axon is filled with a mixture of high, medium and low electron dense granules. However, the number of NS granules in these cells were much lower than those found *in vivo*. It remains to be determined whether this decrease is due to release of NS material or to a decrease in the synthesis of NS material *in vitro* or a combination of both.

Electrophysiological techniques provided additional information on the functional state of the NS cultures. Unit electrical activity was observed in numerous NS cells. This activity was spontaneous and endogenous in nature and was comparable to similar activity *in vivo* (see discussion). Action potentials taken from cultures incubated for a few days did not differ significantly from those of older cultures. The frequency of discharge of single units ranged from 0.2/sec to 2.3/sec. The duration of individual action potentials was variable and long and lasted from 2 to 7 milliseconds (SESHAN *et al.*, 1974).

In vitro analysis of the corpus cardiacum

The extensive nerve fiber outgrowth from the whole surface of the corpus cardiacum or from small segments of the organ (Fig. 3) is a prominent feature of this gland. Inspection of a large number of cultures with inverted and interference microscopes showed that these fibers resemble those found in cultures of the embryonic nervous system and have morphological features of true nerve fibers. Their identification as true axons was confirmed by the silver technique which stains them either deep brown or black. Aside from the neuronal processes, numerous cells of different types migrate out of the explant. One type of cells found in large numbers is small and spindle-shaped and has been identified as glial cells. In Fig. 3 these cells at this magnification are hidden among the fibers (small closed arrows). A few cells larger in size with neuronal features (long arrows) suggestive of neuroglandular cells are present in the migratory zone. A third cell type (small open arrow) seldom found in CC cultures resembles the cells of the tracheal system.

In long term cultures (4–8 week old), the CC and embryonic explants present in the same culture vessel establish contact to one another with neuro-fibrillar connections. Contractility of foregut and heart explants become evident during this period and this activity persists as long as both these sets of explants are in intimate neuro-fibrillar contact with each other.

The intercellular spaces of the corpus cardiacum of 4–8 week old cultures are rich with NS granules after staining with GOMORI and others' methods and only few such granules are evident in the axons. The electron microscope provided additional evidence for the presence of numerous electron dense granules in the cell pericarya and axons which is suggestive of NS activity *in vitro* (Fig. 4).

Using the same electrophysiological techniques as those used for NS cultures, one-third of the CC cultures showed bioelectric activity (SESHAN *et al.*, 1974). The firing pattern of the CC was very irregular and contrasted sharply with those of the NS cultures. The mean firing rate of 29 single units was 1.88 spikes/sec (range 0.28–4.37/sec). Many units displayed a bursting pattern of firing in which the interburst interval of individual units averaged 13 sec (range 2–25 sec). The CC units rarely fired continuously with regular inter-spike intervals as those of the NS cells.

In vitro analysis of the corpus allatum

Cultures of nymphal corpus allatum produce a few protoplasmic filaments and

small spindle-shaped cells are found associated with the filaments. It is not certain whether these cells and fibers originate from the CA or from CC tissue which extends to this organ. When CA–CC is cultured together with other tissues, the cells and nerve fibers produced by the CC envelop the CA to such an extent that it is difficult to determine whether the CA contribute to these activities. In serial sections of the cultured CA stained with hematoxylin and toluidine blue techniques, the cells were either closely packed together or in some cases they were spread apart. NS granules were sparingly distributed among the cells. At the electron microscope, NS axons filled with electron dense granules were present among the cells (Fig. 5).

The production of juvenile hormone by the corpus allatum was studied in radiotracer experiments. The known juvenile hormones are acyclic sesquiterpenes (Fig. 6). L–methionine is efficiently used as a donor of the ester methyl group *in vivo* (METZLER *et al.*, 1971; 1974) and *in vitro* (JUDY *et al.*, 1973). This procedure enabled us to detect and quantify the hormones secreted by the corpus allatum when methyl–^{14}C–methionine had been added to the culture medium.

The time course of incorporation of methyl–^{14}C–methionine into juvenile hormone indicated a high rate of JH production by adult cockroaches of both sexes (approximately 10 ng/day/CA pair). Females carrying fully developed ootheca produced nearly four to five times the amount of JH in contrast to those without ootheca during the first week of incubation. Toward the end of the second and third week, JH production declined sharply and was undetectable after the fourth week. The hormone was identified as JH III (Fig. 6). There was no evidence for the production of other juvenile hormones, e.g. JH–I or JH–II. Corpora allatta of mature nymphal specimens (10th–12th instar) of both sexes also produced JH–III exclusively, but the amounts recovered were about ten times lower than those of adult glands. The addition of mevalonolactone to CA cultures increased the JH–III yield upto ten-fold. Addition of homomevalonolactone had no effect.

DISCUSSION

The successful *in vitro* culture and long-term maintenance of the cockroach neuro-endocrine organs have shown the considerable flexibility and adaptability of these structures in a synthetic chemically defined medium. Further, the results obtained by these techniques have opened new lines of investigations which were not accessible previously. The ability of fully differentiated nymphal and adult cockroach neuro-endocrine organs to produce neuronal fiber processes and at the same time display neurosecretory granules in them at the optic and ultrastructural levels demonstrates the dual attributes of these tissues. Moreover, the capacity of the NS and CC cultures to generate bioelectric potentials *in vitro* adds physiological evidence for their functional integrity. Electrical properties of NS tissues first analyzed in vertebrate systems (BERN & YAGI, 1965) have recently been investigated in invertebrate groups and in particular in insects (MADDRELL, 1974). However, to our knowledge, no attempts have been made to test these properties in insect systems *in vitro*. Our *in vitro* techniques lend themselves to detailed investigations in this field.

The high concentration of cysteine/cystine-rich peptides in the NS cells has been utilized for biosynthetic studies (see review by MADDRELL, 1974). In these *in vivo* tracer studies type "A" NS cells are labeled preferentially in contrast to other NS or non-NS neurons. STEEL & MORRIS (1975)

using X-ray microanalytical techniques reported a more direct measurement of NS activity in *Rhodnius prolixus*. We did not find any preferential incorporation of ^{35}S–cysteine in our *in vitro* studies of the NS cells. These investigations now in progress will tell whether NS cells synthesize, transport and release specific proteins *in vitro*.

The significance of the effect of embryonic organs or ovarian follicles cultured together with NS and CC cannot be underestimated. While it is well known that the neuro-endocrine glands *in vivo* release a variety of hormones into the circulation which affect the target organs, the reciprocal effects of target organs upon the neuro-endocrine system are not clearly understood, at least in insects. Our observations that neuro-endocrine organs atrophy *in vitro* in the absence of target tissues raises the question whether the embryonic and ovarian tissues release any specific agents into the medium which enhance the survival of the neuro-endocrine organs. The release of a 'foregut' factor by cultured segments of embryonic foregut promoting the long-term survival of dissociated embryonic nerve cells has been suggested in earlier studies (CHEN & LEVI-MONTALCINI, 1970). Equally important is the question whether the neuro-endocrine organs synthesize and release hormones or hormone-like factors promoting further differentiation of the embryonic organs and ovarian follicles with which they establish neuro-fibrillar connections.

The capacity of the CA of nymphs and adults of *P. americana* to produce juvenile hormone *in vitro* supplements the morphological data that these glands are in fact quite active *in vitro*. The CA of other cockroach species (*Periplaneta fuliginosa* and *Blaberus discoidalis*) *in vitro* also produce JH-III (DAHM *et al.*, 1976). The only JH found in these cultures is JH–III, which is in agreement with the results of other workers on this and other hemimetabolous species (JUDY *et al.*, 1973; MULLER *et al.*, 1974; PRATT *et al.*, 1975). JH–I and JH–II have been detected in only one orthopteran species. LANZREIN *et al.*, (1975) demonstrated the presence of all the three JH homologues in blood samples of nymphs and adults of the cockroach *Nauphoeta cinerea*. Otherwise JH–I and/or JH–II was produced by CA *in vitro* by lepidopteran insects (DAHM *et al.*, 1976; JUDY *et al.*, 1973; ROLLER & DAHM, 1968; 1970; 1974). Whether one or more of these hormones are specialized for morphogenetic or gonadotropic functions in the living insect is still a matter of controversy. Nevertheless, we hope, studies along these lines in combination with other techniques on the cockroach and other insects will help to illuminate the complex and diversified roles of the neuro-endocrine glands in the life of insects.

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STUDIES ON THE POLYMORPHISM OF ∝- ESTERASE ACTIVITY IN A FEW MEMBERS OF *NASUTA* SUBGROUP (GENUS: *DROSOPHILA*)

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Polyacrylamide gel electrophoretic technique was used to assay the activity of ∝-esterases in various members of *D. nasuta* subgroup. Five zones of activity designated as EST A to E were recognized. Further 1 to 5 bands in each zone were observed in these species under study. Both similarities and dissimilarities with regard to degree of variation in the isozymes compared with the presumed degree of relationship established by cytological and hybridization studies are discussed.

INTRODUCTION

The *nasuta* subgroup of the *immigrans* species group of the subgenus *Drosophila* consists of a cluster of sibling species. Though WILSON *et al.*, (1969) included eight biologically valid species in this subgroup, the detailed cytogenetical studies including the hybridization experiments made by NIRMALA & KRISHNAMURTHY (1973–1974), RANGANATH & KRISHNAMURTHY (1976) and RANGANATH *et al.*, (1974) enabled them to establish three morphophenotypic complexes on the basis of the extent of silvery markings on the frons. The first is the frontal sheen complex with silvery sheen on the entire frons, and includes *Drosophila nasuta nasuta*, *D. n. albomicana*, *D. n. kepulauana* and *D. kohkoa*. The second is the orbital sheen complex with silvery markings confined to sides of the orbits and include *D. sulfurigaster sulfurigaster*, *D. s. bilimbata*, *D. s. albostrigata*, *D. s. neonasuta*, *D. nixifrons* and *D. pulaua*, while the third includes *D. pallidifrons* without any such markings. On the basis of hybridization experiments and the fixed inversion differences detected between members of the *nasuta* subgroup, RAJASEKARASETTY *et al.*, (1975) were able to establish certain interrelationships between the above said members under study. NAIR *et al.*, (1971) by their studies on the isozyme polymorphism in six members of *mesophragmatica* group of *Drosophila* have demonstrated that there is a correlation between the cytotaxonomic relationship and the isozyme pattern. As the taxonomic status of the original strain of *D. nasuta* in relation to other members of *nasuta* subgroup has been established, the authors wanted to evaluate whether there is any similarity between the earlier cytogenetic findings and the isozyme differences between the above members. Molecular manifestation in the form of isozyme activity by electrophoretic assay has been utilized by several workers to estimate the genetic similarity or dissimilarity between populations or species. JOHNSON *et al.*, (1966) studied esterase differences between taxonomically different species and found a close agreement in the patterns. HUBBY & THROCKMORTON (1968) compared seven isozymes with nineteen systems in triads of closely related species in *Drosophila*

and found that sibling species shared isozymes with identical electrophoretic mobility about 50% on the average and lesser degree of sharing occurred between more distantly related ones. KANAPI & WHEELER (1970) have made preliminary attempt to detect the isozyme differences among few members of the *nasuta* subgroup at a time when the original strain of *D. nasuta* from Seychelles islands was not available to them and thus the cytogenetic status of the then available members were yet to be analysed. As the taxonomic status of the original strain of *D. nasuta* has been established (NIRMALA & KRISHNAMURTHY, 1973; RANGANATH *et al.*, 1974) the present studies on the molecular differentiation in terms of ∝-esterase isozymes were undertaken and the results are presented here.

MATERIALS AND METHODS

Different members of *D. nasuta* subgroup used for the present analysis of esterase isozyme activity, along with their geographic origin are listed in Table I.

TABLE 1. Members of *D. nasuta* subgroup used in the present study along with their geographic origin

1.	*D.n. nasuta*	Coorg (Karnataka, India)
2.	*D.s. neonasuta*	Coorg (Karnataka, India)
3.	*D.n. albomicana* (3146.3)*	Chi-tou, (Taiwan)
4.	*D.n. kepulauana* (3121.3, no. 2)*	Sarawak
5.	*D. Kohkoa* (3256.3, no. 1)*	Gulf of Thailand
6.	*D. pulaua* (3121.5)*	Sarawak
7.	*D.s. sulfurigaster* (3019.8)*	Wau, (New Guinea)
8.	*D.s. albostrigata* (3261.2)*	Mt. Makelins, (Laguna)

* Stocks received from Genetics Foundation, University of Texas at Austin, Texas, U.S.A. The stock number is mentioned in brackets.

Assays were made for ∝-esterases (EST) using polyacrylamide gel electrophoretic technique of DAVIS (1964), with the following modifications. Homogenates of three day-old single males constituted the material for assay of izozymes. Five stock solutions A, B, C, D and E were prepared following the procedure of Davis (1964). Small pore gel was prepared by mixing one part of solution A, two parts of C, one part of distilled water and four parts of 0.14% ammonium persulphate. This mixture was allowed to polymerise in cylindrical glass tubes in the presence of sunlight. Then the large pore gel solution was prepared by mixing one part of solution B, two parts of D and one part of solution E and layered to a height of 5 mm over the small pore gel. The large pore gel solution was allowed to polymerise in the sunlight. The gel tubes were placed in containers where the basal portion carrying small pore gel was immersed in tray buffer (Boric acid Sodium hydroxide buffer of 0.3M and pH 8.65). The sample homogenate mixed with 0.2 ml of 40% sucrose solution was layered over the large pore gel. This was further layered with the tray buffer in such a way as to completely conceal the electrodes. Electrophoresis was carried out at 4°C with 80 volts current, for 2 hours. Afterwards, the gels were removed from the tubes and were incubated in the staining solution at 37°C. After the enzyme bands appeared the gels were fixed in 7% acetic acid.

The sites of esterase isozymes were stained by the coupling of ∝-naphtol with a diazonium salt (Fast blue RR salt). Here ∝-naphthol was liberated by the activity of esterases from ∝-naphthyl acetate which was added as the substrate.

25 mg of ∝-naphthyl acetate was dissolved in 1 ml of acetone and 1 ml of water and was added to 12.5 ml of 0.1 M phosphate buffer of pH 5.9, to which 25 mg of Fast blue RR salt was added. The whole solution was mixed thoroughly and added to 12.5 ml of 0.1M phosphate buffer of pH 6.5. This constituted the stain.

RESULTS

A comparative study of various zymograms for ∝-esterase activity manifested by different species of *D. nasuta* subgroup under study has revealed the presence of 1 to 5 zones of activity designated here EST A to E. In all species all the zones of activity are not encountered. The number of bands included in each zone of activity ranges from one to five. The variable positions of the bands on the gel are shown in Fig. 1.

The zone A is nearest to anode, while zone E is close to the origin. The E and D zones have one band each. *D. s. albostrigata* has both the zones D and E while *D. pulaua* has only E. Of the two bands which compete to reach the anode, one runs along with the indicator bromophenol blue and this band occupies a position approximately equidistant from the two poles.

broad bands in the C zone and one band in the B zone are seen. In *D. kohkoa* also we recognise two broad bands in C and one band in B but the positions of these bands differ from the previous species. In the case of *D. pulaua*, there is a single band in the zone E, three broad bands in C and one in zone B. In *D. s. sulfurigaster*, there are three broad bands in zone C and two

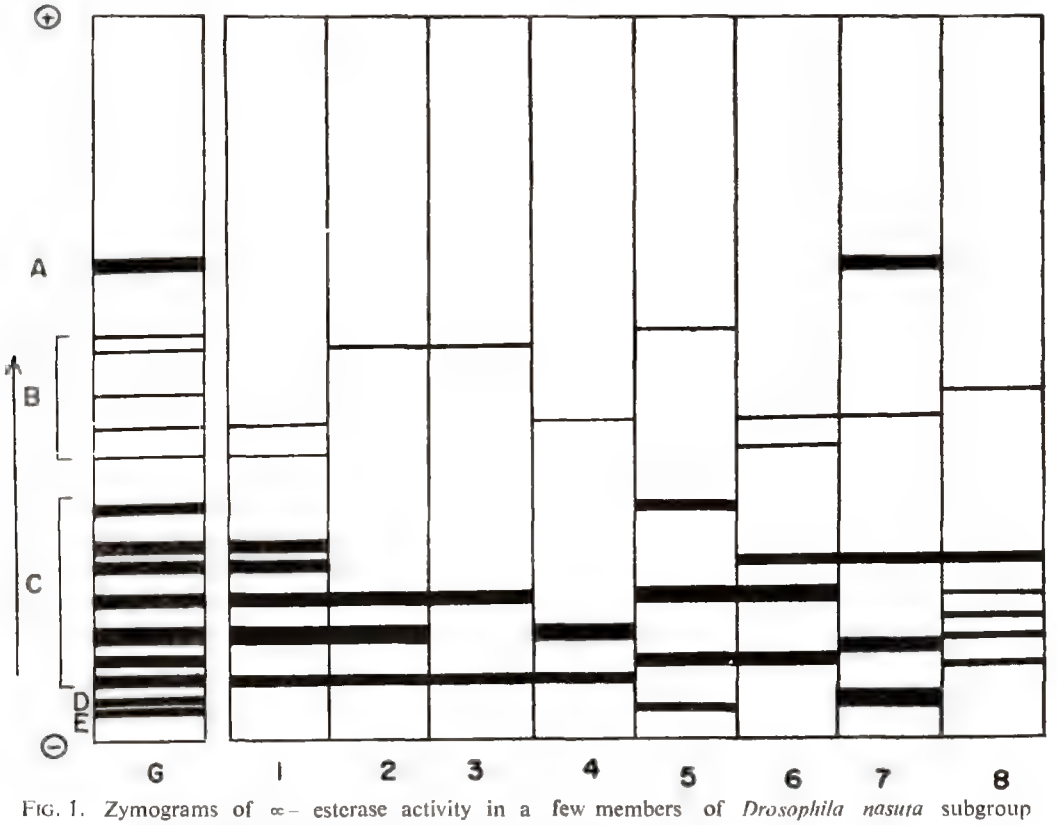

FIG. 1. Zymograms of ∝– esterase activity in a few members of *Drosophila nasuta* subgroup (G) Composite presentation of the five primary zones of activity (1) *D. n. nasuta* (2) *D. n. albomicana* (3) *D. n. kepulauana* (4) *D. kohkoa* (5) *D. pulaua* (6) *D. s. sulfurigaster* (7) *D.s. albostrigata* (8) *D.s. neonasuta.*

In the case of *Drosophila nasuta nasuta,* the A, D and E zones are absent, while the B zone is represented by two bands, zone C is represented by five broad bands. In the case of *D. n. albomicana* there are three broad bands in C zone and only one band in the B zone, while in *D. kepulauana* two bands in zone B. *D. s. albostrigata* exhibits all the five zones of activity, where zones A, B, D and E have one band each and the zone C is represented by two broad bands. The band that moves faster than the dye towards the anode is EST A and is found in 30% of the individuals of this species.

Further, no other species carries this band. *D. s. neonasuta* has one band in B zone and zone C is represented by one broad band and four thin bands.

DISCUSSION

In addition to cytogenetic methods, isozyme studies have been used to study gene differences within Mendelian populations as well as between species. Further, a correlation between classical cytotaxonomic studies and isozyme studies is found to be more meaningful. AYALA & POWELL (1972) have studied enzyme variability in sibling species of the *willistoni* group and discussed the relevance of their results in differentiation of these species.

Of the eight members employed in the present analysis. the hybridization experiments have shown that *D. n. nasuta, D. n. albomicana, D. n. kepulauana* and *D. kohkoa* belong to the frontal sheen complex; RANGANATH *et al.*, (1974) have shown *D. n. albomicana* as a chromosomal race and *D. n. kepulauana* as a semispecies of *D. n. nasuta.* Further, *D. n. albomicana* is genetically closer to *D. n. nasuta* than to other members of the group because the hybrids are viable and fertile. But *D. n. kepulauana* due to its partial reproductive isolation from *D. n. nasuta* might have undergone a higher level of genetic divergence, while *D. kohkoa* has attained the status of a species because the cross does not give viable and fertile hybrids. Examination of the α– esterase patterns of all the species under discussion, as revealed by the zymograms confirm the findings through hybridization experiments. The banding pattern of the members *D. n. nasuta, D. n. albomicana* and *D. n. kepulauana* share some common features particularly in the zone C. The nature of banding of *D. kohkoa* differ from *D. n. albomicana* and *D.n. kepulauana* in this zone, but it possesses similarities with that of *D. n. nasuta* in the zones B and C. Further, among the members of the *sulfurigaster* complex, *D. s. sulfurigaster, D. s. albostrigata* and *D. s. neonasuta* have different levels of reproductive isolation reflecting the extent of their genetic affinities. But *D. pulaua* is completely reproductively isolated from these members (NIRMALA & KRISHNAMURTHY, 1973–74; RANGANATH & KRISHNAMURTHY, 1976). *D. s. albostrigata* is the only species which has exhibited activity in all the five zones. It is interesting to note that none of the bands of *D. s. neonasuta* are similar to the bands of *D. s. sulfurigaster*. The zymograms of *D. s. sulfurigaster* and *D. s. albostrigata* present the occurrence of similar activity in zones B and C. The nature of banding of *D. pulaua* has revealed varying degrees of similarities with *D. s. sulfurigaster* and *D. s. albostrigata* but it completely differs from *D. s. neonasuta.*

Thus the extent of genetic differences as revealed by the α – esterase activity in the members of *nasuta* subgroup lends some support to the earlier findings of hybridization experiments. In view of these findings it is felt that one has to employ both cytogenetic as well as isozyme analyses to explore the exact genetic relationships of different species.

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STUDIES ON THE NUCLEAR POLYHEDROSIS OF *PERICALLIA RICINI* F. (LEPIDOPTERA : ARCTIIDAE)

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Studies on the nuclear polyhedrosis of the larvae of *Pericallia ricini* F. (Arctiidae) revealed that the infected larvae exhibited all the typical symptoms of nuclear polyhedrosis. Caterpillars of second, third and fourth instars were highly susceptible, those of the fifth instar moderately so and those of sixth instar highly resistant to the infection. The total haemocyte count decreased in the virus-infected larvae progressively from 48 hours after ingestion of the virus. The polyhedra measured 1284.6 ± 12.48 mμ in diameter. They were completely soluble in weak solutions of NaOH, KOH and Na_2CO_3. The thermal inactivation point of the virus was between 90° and 95°C. Exposure of the polyhedra to direct sunlight for 96 hours substantially reduced its infectivity. But it remained highly infectious after exposure to 35°C in an oven for 96 hours, though in 120 hours it lost its infectivity. It thus appeared that in addition to temperature, perhaps light was also responsible for deactivation of the virus under field conditions. The virus was not infective to four species of alternate caterpillars tested.

INTRODUCTION

The black hairy caterpillar *Pericallia ricini* F. is a polyphagous pest feeding on cultivated crops like cotton, castor, banana, cucurbits, pulses and sesamum. JACOB *et al.*, (1972) reported a nuclear polyhedrosis in this insect. Information gathered on the nature of the pathogen and on the host–pathogen relationships are presented in this paper.

MATERIALS AND METHODS

The larvae used in these studies were reared in the laboratory on castor (*Ricinus communis* L.) leaves. A purified concentrated suspension of polyhedra isolated from the diseased larvae of *P. ricini* and diluted to contain 33×10^7 polyhedra per ml of distilled water and containing 0.1 per cent teepol as wetting agent, formed the infective material. Larval inoculations were done by the spot feeding technique (JACOB, 1972). The polyhedral suspension (5μl) was applied to each spot and the larvae which had consumed the entire leaf area at the spots in 4 to 6 hours, were transferred to fresh uncontaminated foliage individually in sterile plastic cups. Control larvae were fed similarly on spots of distilled water containing 0.1 per cent teepol only.

Susceptibility of the larvae under different instars was assessed as indicated by JACOB & SUBRAMANIAM (1972). Haemocyte counts of the infected larvae were made following the method of SHAPIRO (1967). Statistical 't' analysis was used to compare the differences between means. Dissolution of polyhedra in alkalies was studied by the method of PAWAR & RAMAKRISHNAN (1971). Thermal inactivation point, effect of sunlight on the infectivity and survival of the virus at the highest field temperature were studied as described by LATHIKA & JACOB (1974 c); in the case of survival at the highest field temperature, the polyhedral films were exposed to 35°C in an oven.

Cross transmission to alternate species of lepidopterous larvae was determined by feeding them for 24 hours on their host plant leaves contaminated with the polyhedral suspension.

RESULTS

Symptomatology

The infected second and third instar larvae turned pale 2 to 3 days after ingestion of the virus—a feature not shown by the later instars at this stage. The larvae became lethargic, showed reduced feeding

TABLE 1. Susceptibility of different instars of the larvae of *P. ricini* to infection by NPV.

Instar of larva	No. of larvae inoculated	Incubation period (days)		Per cent larval mortality* due to		Pupation %	Pupal mortality
		Range	Mean	Polyhedrosis	Other causes		
II	50	4-7	5.2	100	—	—	-
III	50	4-8	5.9	92	8	—	—
IV	50	4-10	7.0	92	8	—	—
V	50	6-9	8.2	72	12	16	—
VI	50	8	8.0	8	—	92	—

* There was no mortality due to virus in control.

and finally stopped feeding 2 to 3 days prior to death. Some of the larvae discharged a dark brown fluid through their mouth. In advanced stages of infection the cuticle became very fragile and ruptured readily on touch or by movements, liberating the liquefied body contents containing millions of polyhedra. Death occurred in 4 to 8 days after ingestion of the virus. The cadavers were found either hanging head downwards or lying flat on the leaf or other surfaces.

The body fluid which was clear in the initial stages turned turbid as the infection advanced. Dissection of the infected larvae showed that the fat body was opaque white in appearance.

Larval susceptibility

Results presented in Table 1 show that, as the stage of the larvae at inoculation advanced there was a decrease in the mortality caused by the virus infection and a prolongation of the incubation period. Those larvae which survived the infection when inoculated in the fifth and sixth instars reached the adult stage normally. Thus the 2nd, 3rd and 4th instar larvae showed high susceptibility to the virus infection. Fifth instar also showed fairly high susceptibility with 72 per cent mortality, the sixth instar was however, highly resistant.

Total haemocyte count

Fig. 1 illustrates the changes in the average number of circulating haemocytes (THC) in healthy and virus infected larvae. There was no significant difference between the healthy and virus infected larvae in their THC at 24 hours after inoculation. At all subsequent intervals the diseased larvae had significantly fewer haemocytes. Further, in

TABLE 2. Effect of different alkalies on polyhedra of *P. ricini*.

Time given for dissolution (minutes)	NaOH (%)		KOH (%)		Na_2CO_3 (%)	
	0.1	0.2	0.1	0.2	5.0	10.0
1	+		+	—	+	—
2	+	—	+	—	+	—
3	+	—	+	—	+	—
4	+	—	+	—	+	—
5	+	—	+	—	—	—
10	+		+	—	—	—
15	—	—	—	—	—	—

\+ Polyhedra present.
— Polyhedra absent.

healthy larvae there was a steady increase in THC with age while in the infected ones there was a steady decrease.

Size and shape of polyhedra

Electron micrograph of polyhedra (Fig. 2) showed that they were irregular in shape and varied considerably in size. The diameter ranged from 943.6 $m\mu$ to 1829 $m\mu$ with an average of 1284.6+12.48 $m\mu$.

Alkali resistance of polyhedra

It may be seen from Table 2 that 0.2 per cent KOH or NaOH dissolved the polyhedra within 2 minutes while 0.1 per cent solution of either alkali required more than 10 minutes to produce the same effect. In solutions of Na_2CO_3 the polyhedra dissolved in 5 minutes in 5 per cent and in 2 minutes in 10 per cent solutions.

Thermal inactivation point of the virus

The results (Table 3) reveal that infectivity of the virus was not affected by exposure to a temperature of up to 70°C for 10 minutes, but the infectivity started declining when the temperature was raised to 80°C and above. The virus did not show

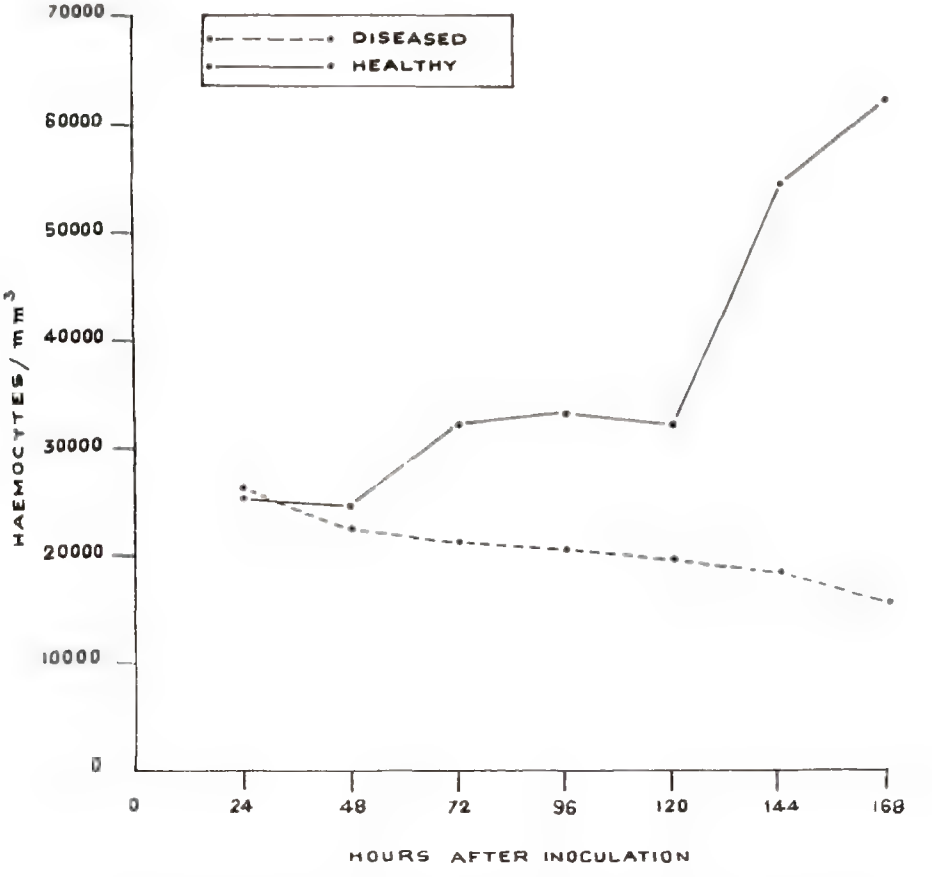

FIG. 1. Average number of circulating haemocytes in healthy and NPV infected larvae of *P. ricini* at different intervals after inoculation.

FIG. 2 Electron micrograph of polyhedra isolated from NPV infected larvae of *Pericallia ricini*. 29675 x.

any infectivity when subjected to a temperature of 95°C. These indicate that the thermal inactivation point (TIP) of the virus lay between 90° and 95°C.

Survival of the virus under the highest field temperature

The data presented in Table 4 show that exposure of the polyhedra to 35°C upto

TABLE 3. Effect of different temperatures on the infectivity of the NPV of *P. ricini* when exposed for 10 minutes.

Temperature °C	No of larvae inoculated	Incubation period in days (Mean)	% Larval mortality due to		Pupation %	Pupal mortality %
			Polyhedrosis	Other causes		
60	50	6.9	100.0	—	—	—
70	50	7.3	100.0	—	—	—
80	50	7.4	84.0	—	16.0	—
90	50	—	32.0	4.0	64.0	—
95	50	—	—	4.0	96.0	—
100	50	—	—	—	100.0	—
Control (without virus)	50	—	—	—	100.0	—
Control (untreated virus)	50	6.0	100.0	—	—	—

TABLE 4. Infectivity of NVP of *P. ricini* exposed to 35°C for different periods.

Duration of exposure to 35°C (hours)	No. of larvae inoculated	Incubation period in days (Mean)	% Larval mortality due to		Pupation %	Pupal mortality %
			Polyhedrosis	Other causes		
12	50	6.1	100	—	—	—
24	50	6.2	96	4	—	—
48	50	6.8	96	4	—	—
72	50	7.8	92	8	—	—
96	50	9.2	88	12	—	—
120	50	10.3	12	4	84	—
Control (without virus)	50	—	—	2	98	—
Control (Untreated virus)	50	6.1	100	—	—	—

96 hours did not significantly affect the infectivity of the virus. But exposure for 120 hours substantially reduced the infectivity and 84 per cent of the larvae pupated normally. There was also a gradual increase in the incubation period of the virus with the increase in the period of exposure to the temperature.

Effect of sunlight on the infectivity of the virus

It is clear from the data presented in Table 5 that the virus remained highly infectious upto 72 hours of exposure to sunlight, though there was a prolongation of the incubation period. The virus however, lost its infectivity with further increase in exposure period. Thus infectivity was reduced to 36% when the polyhedra were exposed to sunlight for 96 hours and it was almost lost after exposure for 120 hours.

Cross infectivity

Results of cross transmission studies reported in Table 6 show that the NPV of *P. ricini* was not infective to any of the 4 species of caterpillars under study.

DISCUSSION

The inverse relationship between larval age and susceptibility to nuclear polyhedrosis infection observed in the present studies has been reported by several earlier workers (TANADA, 1956; MORRIS, 1962; JACOB & SUBRAMANIAM, 1972). It is a case of

TABLE 5. Effect of exposure to sunlight for varying periods on the infectivity of the NPV of *P. ricini*.

Duration of exposure (hours)	No. of larvae inoculated	Incubation period in days (Mean)	% Larval mortality due to		Pupation %	Pupal mortality %
			Polyhedrosis	Other causes		
12	50	6.5	100	—	—	—
24	50	7.0	96	4	—	—
48	50	7.5	96	4	—	—
72	50	9.9	92	8	—	—
96	50	10.3	36	16	48	—
120	50	10.5	8	8	84	—
Control (without virus)	50	—	—	—	100	—
Control (Untreated virus)	50	6.2	98	2	—	—

TABLE 6. Infectivity of NPV of *P. ricini* to different alternate hosts.

Alternate host insect	Stage of larvae at inoculation	No. of larvae tested	% Larval mortality due to		Pupation (%)	Pupal mortality (%)	Infectivity
			Polyhedrosis	Other causes			
Achoea janata	3rd instar	30	0	0	100	0	Nil.
Spodoptera litura	4th instar	30	0	0	100	0	Nil.
Glyphodes marginata	3rd instar	20	0	10	90	0	Nil.
Euproctis fracterna	3rd instar	50	0	5	95	0	Nil.

maturation immunity which according to IGNOFFO (1966 a) is partly due to the normal increase in body weight of the host which might dilute a constant viral dose.

The observation that virus infection causes a decrease in the THC of the infected larvae is in agreement with those in *Heliothis zea* (SHAPIRO *et al.*, 1969) *Spodoptera litura* (JACOB, 1972) and *Spodoptera mauritia* (LATHIKA & JACOB, 1974 b). JACOB (1972) attributed this to the destruction of haemocytes and interference in mitotic division of the blood cells by the virus infection. Haemocytes are known to be one of the major sites of infection by NPV.

In common with other polyhedral viruses, the polyhedra of *P. ricini* also dissolve in solutions of NaOH, KOH and Na_2CO_3. It is known that the degree of resistance towards different alkalies varies with polyhedra from different polyhedroses. In its reaction towards NaOH, KOH and Na_2CO_3 the polyhedra of *P. ricini* closely resemble those of *S. mauritia* (LATHIKA & JACOB, 1974 a). These two polyhedra are less resistant to Na_2CO_3 than other reported polyhedroses such as those of *Pterolocera amplicornis* (DAY *et al.*, 1953) and *Diacrisia obliqua* (JACOB & THOMAS, 1974).

The TIP of NPV of *P. ricini* is seen to be between 90° and 95°C. This agrees with those reported for *S. litura* (PAWAR & RAMAKRISHNAN, 1971) and *S. mauritia* (LATHIKA & JACOB, 1974 c). However this exceeds the general 80°C limit reported for other inclusion body viruses (BERGOLD, 1958; AIZAWA, 1963; HUGER, 1963).

It has been reported that higher field temperatures (35°—45°C) may affect viral stability and viral multiplication (BIRD, 1955; THOMPSON, 1959; IGNOFFO, 1966 b). But the present studies show that the NPV of *P. ricini* can withstand continual exposure to 35°C for 96 hours without losing its infectivity though the virulence started declining on exposure beyond 96 hours. Further, the results presented show that exposure of the polyhedra to direct sunlight for periods up to 72 hours does not affect the viral stability and infectivity. CANTWELL (1967) observed that the NPV of *Trichoplusia ni* is completely inactivated by exposure to direct sunlight for 3 hours. Similarly BULLOCK (1967) also found that *Heliothis* virus applied to cotton foliage loses most of its infectivity after one day and this was attributed partly to the action of ultraviolet rays in the sunlight. LATHIKA & JACOB (1974 c) found that NPV of *S. mauritia* can withstand exposure to sunlight for 72 hours. It thus appears that under the tropical conditions as existing in Kerala, the viruses can withstand exposure to sunlight for longer periods probably due to the difference in the composition of sunlight.

Further, the observation that the virus can withstand exposure to a higher temperature of 35°C for 96 hours while it can stand exposure to sunlight only for 72 hours indicates that under field conditions temperature alone may not be the factor responsible for inactivation of the virus. In a similar study MORRIS (1971) also found that exposure of the NPV of *Lambdina fiscellaria lugubrosa* to 45°C for 200 hours does not affect the final percentage of mortality, but exposure to direct sunlight for 35 hours almost inactivates the virus. Perhaps, temperature along with other factors like ultraviolet radiation may be causing the deactivation.

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BIOLOGICAL STUDIES OF *BRACHYMERIA LASUS* (WALKER) (HYMENOPTERA : CHALCIDIDAE)

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Brachymeria lasus **is a polyphagous parasite attacking the pupal stages of a wide range of pests of crops and vegetables. The adult female lays its eggs one at a time inside the pupae. There are five larval instars. The duration of the development from the egg to adult takes about 10 to 18 days depending upon the weather. Soon after emergence, the female mates. The females live longer than the males. Females predominate on the proportion of sexes. The developmental history, emergence, mating, oviposition, fecundity, nutrition, longevity, sex-ratio and seasonal history of this important species of** *Brachymeria* **are described and discussed.**

INTRODUCTION

The genus *Brachymeria* includes some of the most widely distributed species in the family Chalcididae and is most commonly represented in India by the species *B. lasus* (Fig. 1). *B. lasus* was first described by WALKER in 1841 from India under the name *Chalcis lasus*. It is cosmopolitan in distribution and is a polyphagous species attacking a wide range of pests of crops and vegetables. Its hosts include pests like cotton boll worm *Platyedra gossypiella* S., teak skeletoniser *Hapalia machaeralis* W., rice skipper *Pelopidas mathias* F., the coconut black headed caterpillar *Nephantis serinopa* M. etc. It was in view of the important role of this species as a natural enemy of many pests that the following studies on the biology of *B. lasus* were undertaken.

MATERIALS AND METHODS

Plusia peponis F., *Sylepta derogata* F. etc. were used as hosts for rearing the parasites in the laboratory. The adults were reared in cages (30 cm x 30 cm x 30 cm) with two opposite sides and top made up of glass and the remaining two opposite sides made up of muslin cloth. In each cage approximately 6 females and 3 to 4 males were placed. Glass test tubes (2.5 cm x 15 cm) with their mouths covered with light muslin cloth or cotton plugs were also used for separately accommodating single pairs of female and male parasites. The adults were fed with honey diluted (50%) with water.

RESULTS

Developmental History

The eggs (Fig. 2) are laid one at a time and the egg remains freely inside the body-fluids of the host pupa. Duration of different stages varies depending upon weather. The incubation period varies from 20 to 31 hours. The egg is sausage-shaped with an average length of 1 mm and an average width of 0.2 mm. The cephalic end of the egg is somewhat wider with a small button-like process. The chorion is smooth and hyaline. The yolk granules occupy almost the entire length of the egg leaving only a little space at the two ends.

The first instar (Fig. 3) lasts for a period of 21 to 31 hours. It is typically hymenopteriform with well defined head and 13 body segments. The larva measures about 1 to 1.7 mm in length and 0.2 to 0.3 mm in width. The second instar (Fig. 4) lasts for a period of 17 to 28 hours. It measures 1.7 to 2 mm in length and about 0.4 to

Fig. 1. *B. lasus* : Adult male (Dorsal view).

0.6 mm in width. The third instar (Fig. 5) completes its growth in 20 to 31 hours. It measures 2.6 to 3.5 mm in length and 0.7 to 0.9 mm in width. The fourth instar (Fig. 6) lasts from 22 to 38 hours and measures 3.8 to 5.7mm in length and 1 to 1.8 mm in width. The fifth instar (Fig. 7) is yellowish brown at first and becomes whitish brown later owing to the formation of fatbodies underneath the skin. The duration of the fifth instar varies from 24 to 72 hours. This final instar larva measures 6.6 to 12 mm in length and 2 to 3.5 mm in width.

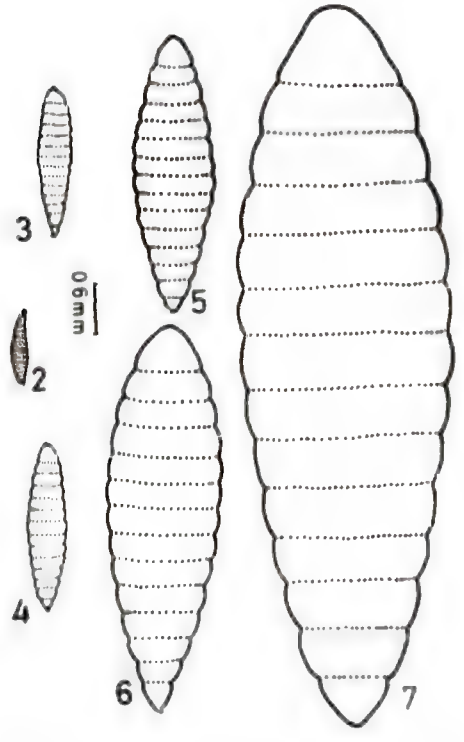
The prepupal stage lasts from 20 to 48 hours from the time the meconia are cast until the last larval skin is shed. The pupa is exarate. The duration of the pupal stage varies from a minimum of 96 hours to a maximum of 144 hours.

Emergence

The process of emergence can be conveniently studied under two heads: Primary emergence and Secondary emergence.

Primary emergence:– When its development is completed the parasite emerges from its own pupa by shedding the pupal exuvium. About a day or a little earlier than this emergence, very slight movements of the tarsal segments of the leg can be seen

through the pupal covering. The movements become more pronounced later on and within 3 to 5 hours before emergence, irregular slits appear on the pupal covering and this is followed by vigorous movements of the hind legs enabling the insect to get free from the pupal covering. The whole process takes a little less than 5 hours.



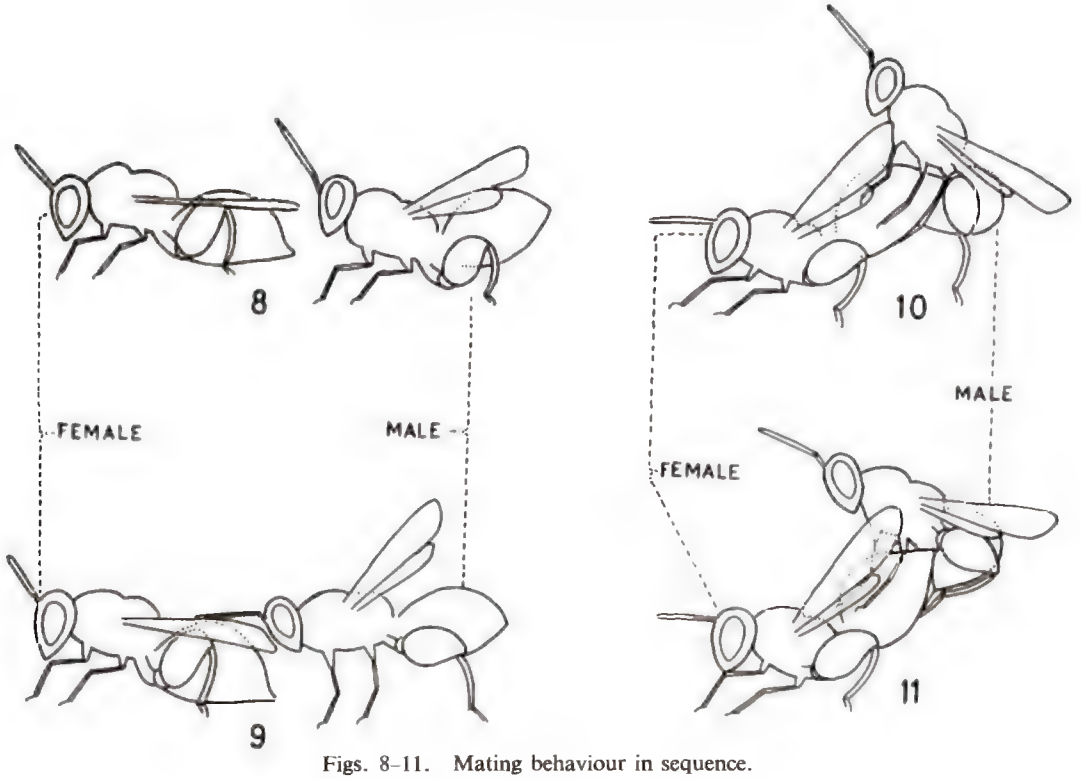
Figs. 2–7. Egg (Fig. 2) and larval stages 1 to 5 (Figs. 3 to 7 respectively). Dorsal view.

Secondary emergence:– After the primary emergence the adult usually remains inside the host pupa for about 12 to 24 hours. Then the chalcid gnaws a round hole usually on the anterior dorsal or ventral side of the thoracic segments of the host puparium. Through this hole the adult parasite emerges out. The secondary emergence of the parasite takes place from 10th to 18th day in the case of female. Since the males have relatively shorter developmental period, they emerge earlier than the females.

Mating behaviour (Figs. 8–11)

A pre-mating period of 1–3 days was observed for the males. Newly emerged males do not generally show interest in the females. The unmated female is ready for mating soon after her secondary emergence.

After the male locates the female, he chases her, while performing the chasing movements the excited male was found swaying its front part of the body from side to side in a characterstic swinging fashion. While the male chases the female, he may stop moving and direct his antennae toward her. He then continues his swaying movements from side to side and approaches her and places his antennae over her wings which are held folded back over her abdomen. The male then vigorously begins to rock the female to and fro and begins to buzz very briefly with his wings simultaneously with a downward pressure at short intervals. Often a sort of "patting" on the head and thorax of the female is carried out by the male with his antennae and for this purpose he moves over to the side or front of the female before taking up his final position behind the female. He then places his antennae on her folded wings as mentioned earlier. When the male begins to rock the female to and fro and to buzz very briefly with its wings as mentioned above, the female may try to escape. If she sits quiet, a sagging of her body towards the substratum occurs. After about a minute or less, she raises her abdomen and the male lowers his abdomen and at the same time mounts over her abdomen in such a way that ventral part of his abdomen is pressed against the undersurface of that of the female. The penis is now thrust forwards and simultaneously the hypopygium of the female's abdomen opens out to receive the penis which is directed into the female's genital opening. During mating the male's tarsi are variously placed. Those of the pro



Figs. 8–11. Mating behaviour in sequence.

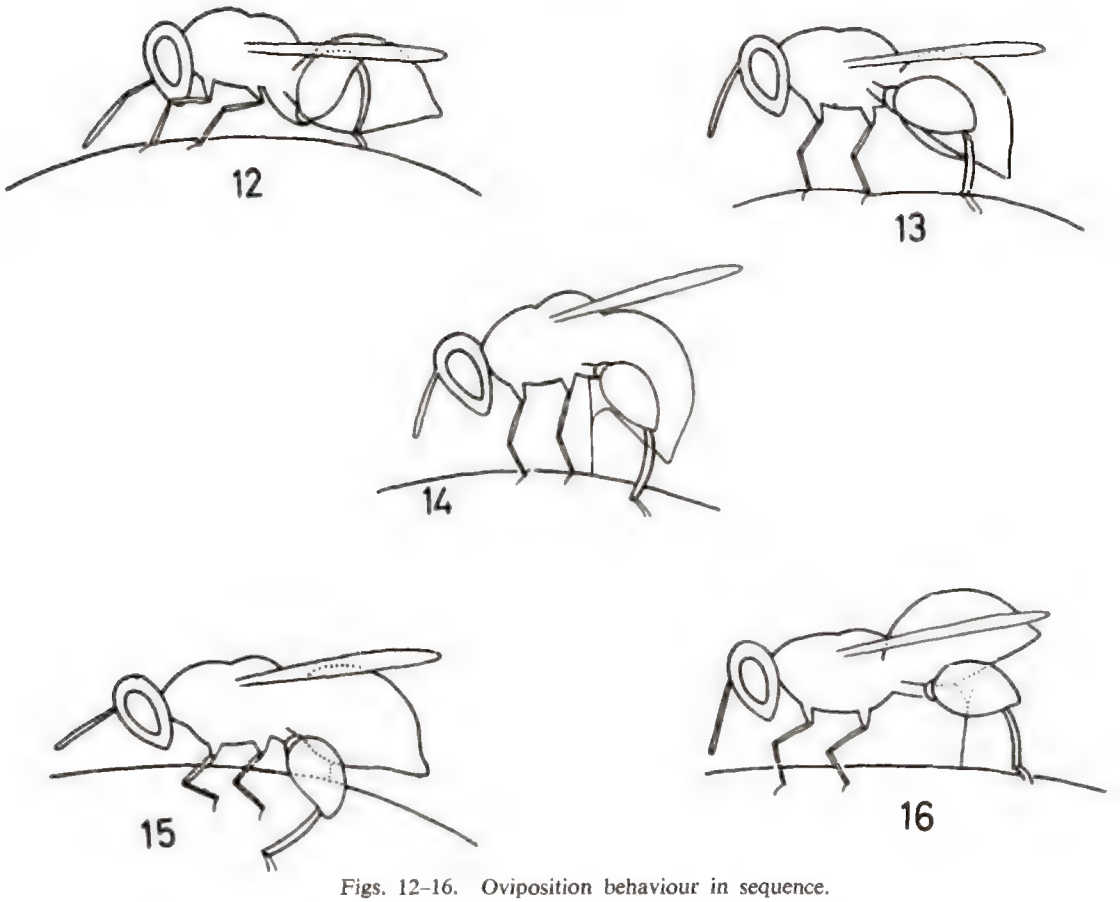
and mesothoracic legs grip some part of the female's wings and abdomen and the metathoracic legs rest on the abdomen of the female or on the substratum. The duration of the act of copulation is about 8 seconds.

Mating usually occurs shortly after the emergence of the female. The adult females which emerge during the night or early morning hours are usually ready for mating by 9 or 9.30 a.m.

Oviposition (Figs. 12–16)

B. lasus is a pupal parasite. As a rule the length of preoviposition period of *B. lasus* is 2 to 5 days. Preliminary to oviposition the parasite makes a thorough examination of the host pupa by means of its antennae. At times during this investigation she may move away from the host, as though dissatisfied, perhaps to return again immediately. Finally after these preliminary processes of examination, she locates a suitable site, usually on the thoracic region of the host, for the penetration of her ovipositor. Females sometimes compete for the same host, the larger or stronger repulsing the other by using her hind legs; two females simultaneously ovipositing on the same host pupa is not an uncommon occurrence.

When the host pupa is removed from its cocoon (which is often silken and thin in the case of *Plusia peponis* and *Sylepta derogata*) and given to the female, after determining the suitable site on the host for oviposition she bends her abdomen in such a way that the tip of the ovipositor touches the spot selected for penetration for her oviposition. Once the ovipositor is in position, the body is straightened and simultaneously the ovipositor pierces the host pupa. The parasite now takes a firm grip with her powerful hind legs on the host. During this position the wings are held folded and the antennae



Figs. 12–16. Oviposition behaviour in sequence.

remain mostly motionless. The pupa writhes and rolls in an attempt to dislodge its attacker, but the parasite firmly braced, shows remarkable ability to hold on to its host until the act of oviposition is over. The 99.7% confidence limits of the population mean value of the duration of oviposition are found to be (in seconds) 55.6 ± 3.8.

When the host pupa is provided with a silken cocoon (as in the case of *Plusia piponis*), after locating a suitable spot for oviposition, the female makes a hole in the silken cocoon with the help of its mandibles, forelegs and antennae. When a small aperture is made, the antennae are inserted through this hole as if to feel the presence of the host pupa inside. The female then withdraws the antennae, moves itself a short distance forwards so that the ventral part of its abdomen is above the prepared hole in the silken cocoon. She then inserts the ovipositor through the hole for effecting oviposition. But, in case the host pupa writhes and rolls violently she withdraws her ovipositor without ovipositing and then inserts her hind legs into the silken cocoon through the hole so as to grasp the pupa firmly. After doing so she inserts her ovipositor into the same hole and oviposits as described earlier. Rarely the parasite may fail to thrust its ovipositor into the host as the ovipositor may slip on the host's cuticle

due to the violent wriggling movements of the host. In such cases the parasite may choose another site for oviposition or may abandon the pupa after a few attempts.

Once oviposition is completed, the female pulls up her abdomen, straightens her legs and withdraws the ovipositor into its sheath. She now spends a little time cleaning her antennae, head or abdomen by means of her legs.

Often after oviposition the female sucks up the fluids oozing out of the host pupa through the puncture made by her for oviposition.

On a few occasions females have been seen ovipositing on pupae of *Sylepta derogata* by thrusting the ovipositor across the silken cocoon without making a hole in it as described earlier.

Fecundity

The female lays only a single egg as a reult of each act of ovipositon. Usually 3 to 6 eggs are laid by a female during the course of a day. The maximum number of eggs laid in a day is 8. The maximum number of eggs laid at a stretch is 5 and in most cases 3 to 4 only. After ovipositing 3 to 5 times, the female has been observed taking a period of rest for about 10 minutes or more before starting the process again.

The number of eggs a female is capable of laying during her life time could not be defenitely ascertained due to difficulties in having a constant supply of host pupae. However, when supplied with enough fresh pupae every day one female laid 131 eggs within her life span of 43 days and another 61 eggs within a short life span of 20 days. This is approximately 3 eggs per day in each case. The average life span of a female is about 53 days and at the rate of 3 eggs per day a female may be capable of laying about 159 eggs in its life. However the maximum realization of this potential will also depend on many other factors like climate, individual variations, availability of hosts etc.

Parthenogenesis

The parthenogenesis in *B. lasus* is arrhenotokous and virgin females produced only males. Mated females produced both male and female progeny. The males produced by virgin females were all found to be normal and fertile.

Nutrition

Adults take sugary fluids like honey, nectar, etc.. and occasionally feed on the body juices of their hosts. In the laboratory they were fed with honey diluted to 50% with water. In nature, as in other chalcids, *B. lasus* feeds on extrafloral nectaries, honeydew, etc. Thus the adults can be observed during the morning hours in bright sunshine, on the extrafloral nectaries of several plants, particularly of *Cassia tora*. In confinement, the females have often been observed feeding on the host fluids oozing out from the oviposition puncture. Occasionally the female parasites were seen stinging the host pupae exclusively for feeding on their body fluids.

Longevity

Usually the females of *B. lasus* have been found to live for a longer period than the males. Though no wide variation in the life span of adults was observed during different seasons of the year in the South Malabar, the adult life span was comparatively a little shorter during the summer months than during the other periods of the year.

Diluted honey (50%) was given as food for the adults reared in the laboratory for the survival was maximum in such honey fed individuals. The life span of 200 such females varied within the range of 21 to

95 days. The distribution of life span is diagramatically represented in Fig. 17. The 99.7% confidence limits of the population mean value are found to be (in days) 53.3±3.8. Under similar conditions the life span of 200 males varied within a range of 15 to 62 days. The distribution of life span is diagramatically represented in

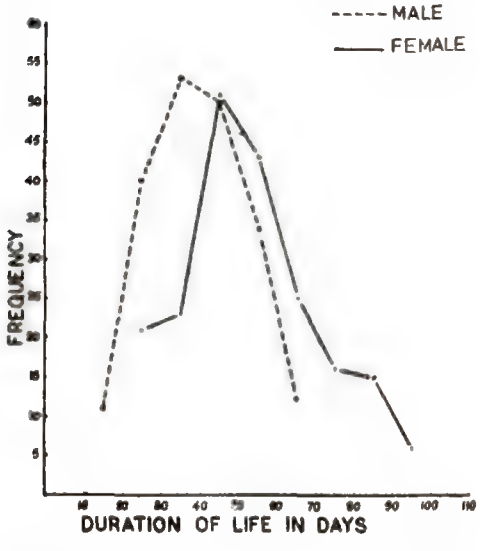


Fig. 17. Frequency polygon showing the distribution of 200 males and 200 females according to their life span (in days).

Fig. 17. The 99.7% confidence limits of the population mean value are found to be (in days) 39.6±2.8.

Sex ratio

Females predominated in the proportion of sexes both in the laboratory-reared individuals and in specimens collected in the field. Out of the total 556 individuals reared in the laboratory during 1970 and 1971, there were 332 females and 224 males. The average sex ratio has been found to be 100 females for 67.5 males. However individuals resulting from parthenogenetic reproduction and individuals from smaller hosts were not included in this sex ratio since virgin females gave rise to only males and smaller hosts tended to produce a high proportion of males. The average sex ratio of adults collected from the field was 100 females for 53 males.

Seasonal history

In the South Malabar (Kerala) adults of *B. lasus* were found occurring throughout the year (1970 and 1971). Their numbers were found maximum during the colder months (especially in September & October). During the remaining part of the year, their numbers were considerably reduced, especially during the monsoon months.

DISCUSSION

In *Brachymeria fonscolombei* (D.) according to PARKER (1924) there is a distinct peduncle at the anterior end of the egg. SYCHEVSKAYA (1966) also noted a distinct peduncle at the anterior end of the egg of *B. minuta* (L.) and at the tip of this peduncle six rounded and shiny projections by which the egg is fixed to the internal tissue of the host. But in the case of *B. lasus* no such peduncle with or without projections is seen for the eggs. DOWDEN (1935) noted that the egg of *B. compsilurae* (C.) has a membrane enveloping the chorion but no such membrane could be observed in the case of *B. lasus*.

The first instar larva of *B. lasus* is hymenopteriform. In certain species of *Brachymeria* like *B. compsilurae* (DOWDEN, 1935), *B. fonscolombei* (PARKER, 1924), *B. minuta* (SYCHEVSKAYA, 1966) etc., instead of hymenopteriform first instar larva, a caudate type first instar larva exists. The larval instars of *B. intermedia* (N.) (DOWDEN, 1935) and *B. lasus* resemble very closely.

B. fonscolombei (D.) manifested no preference for any particular portion of the host for oviposition (ROBERTS, 1933). *B. lasus* was found to give preference to the thoracic

region of the host for oviposition. This is probably because the thoracic region of the pupa is not involved in the writhing movements and also because it is more spacious for oviposition than the tail region of the host pupa. It may be that in the case of *B. fonscolombei* there is no preferred site for oviposition since the host is the larval stage.

B. lasus takes two kinds of nourishment, namely naturally occurring sugary liquids and the body juices of their hosts. *B. lasus* female licks the fluid exuding from its victim's body at the site of penetration of her sting. Such feeding ensures adequate quantities of proteins in addition to the carbohydrates derived from the feeding of honeydew or naturally occurring sugary fluids. It is now well known that such proteinaceous food helps the optimum egg laying capacity.

Because of weather conditions favouring development throughout the year individuals of *B. lasus* are found at all seasons of the year in the South Malabar area where this investigation was carried out.

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STUDIES ON THE INHIBITION OF HONEY BEE CHOLINESTERASE BY CARBAMATES

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The kinetic constants for the inhibition (I_{50}, Ka, k_2, Ki, k_3 and Ka' values) of honey bee cholinesterase by furadan and its two analogues were determined and evaluated. Furadan proved to be a potent inhibitor of honey bee enzyme. All the three carbamates showed a high affinity for honey-bee ChE. The affinity constants ranged from 0.22 to 2.68×10^{-7} M. There was little variation among the carbamylation constants, indicating a minor role for the carbamylation step in the inhibition process. The significant variation in the bimolecular reaction constants of the three carbamates could be attributed to the variations in the affinity constants rather than to the carbamylation constants. The average apparent affinity constants were, in most cases, comparable with the Ka values and they increased progressively with the concentration of inhibitor.

INTRODUCTION

Eventhough many studies have been undertaken on the inhibition of cholinesterase (ChE) by organophosphates, studies undertaken on the enzymic inhibition by carbamates are relatively scanty.

Carbamate hydrolysis follows a scheme similar to that for the normal substrate:

$$E + I \underset{k-i}{\overset{k_i}{\rightleftarrows}} E.I \xrightarrow{k_2} E' \xrightarrow{k_3} E$$

(E = free enzyme, I = free inhibitor, E.I = reversible enzyme-inhibitor complex and E' = carbamylated enzyme).

MAIN & HASTINGS (1966) derived equations for the carbamylation reaction of ChE's:

$$\frac{(I)\,\Delta t}{2.3\,\Delta \log v} = \frac{(I)}{k_2} + \frac{1}{k_2'}$$

where, (I) = inhibitor concentration and $t/2.3\,\Delta \log v$ = first order rate constant at constant (I). Affinity, carbamylation and bimolecular reaction constants in the present studies were derived from the above equation. The rate constants for decarbamylation (k_3) have been calculated according to the first order equation,

$$\ln (E'/Eo) = -k_3 t$$

where E' = carbamylated enzyme, Eo = uninhibited enzyme and t = time in minutes.

A study was also undertaken to find out the influence of substrate concentration on the rate of enzymic hydrolysis under conditions of competitive inhibition according to the linear equation,

$$v = Vm - Km\,(1 + I/_{Ki}).\quad v/S.$$

MATERIALS AND METHODS

Cholinesterase was obtained from the heads of Indian honey bee, *Apis indica* F. and ten heads were used per ml of buffer-saline for homogenization. It was carried out in an all-glass electrically operated POTTER-ELVEHJEM type homogenizer in ice cold Tris-HCl buffer (0.05 M, pH 7.5) containing 1.5 per cent NaCl. The homogenates were centrifuged for 10 minutes at 6000 rpm in a refrigerated centrifuge at O°C. The supernatants were collected in vials and stored at -5°C until used.

Estimations of enzyme activity were done by the method of ELLMAN *et al.*, (1961) as modified by

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SMISSAERT (1964). Acetylthiocholine (ATCh) and dithionitrobenzoic acid (DTNB) used for the assay were the products of Sigma chemical company, St. Louis, Missouri, U.S.A. Furadan and its two analogues, 3-OH carbofuran and 3-keto carbofuran were supplied by the Niagara Chemical Division, FMC Corporation, Middleport, New York, U.S.A. Other chemicals used in the present study were obtained locally and were analytical reagents of the highest purity.

For the determination of I_{50} values, the enzyme (0.1 ml) and inhibitor (0.1 ml) were incubated at 30°C for a period of 30 minutes. Then a reaction mixture of 1.5 ml was constituted by adding to the above mixture 0.3 ml of buffer-saline, 0.5 ml of DTNB and 0.5 ml of ATCh (1×10^{-3} M). A reaction mixture with buffer-saline instead of inhibitor served as control, while one without enzyme and inhibitor was used as blank. The change in the optical density was recorded after an interval of 5 minutes. The I_{50} values were derived from the $e/E^1 - 1/(I)$ graphs (where E^1 = percentage of inhibited enzyme, e = percentage of enzyme remaining active and (I) = inhibitor concentration).

In the experiment to find out the carbamylation, affinity and bimolecular reaction constants, 0.1 ml enzyme was allowed to react with various concentrations of inhibitors in a total volume of 1.3 ml for 30 seconds, after which a 0.2 ml mixture of ATCh and DTNB was added. A reaction mixture with buffer-saline instead of inhibitor served as control.

For reactivation experiment, 0.2 ml of enzyme and 0.2 ml of inhibitor producing about 70 per cent inhibition were incubated at 30°C for half an hour. The enzyme-inhibitor mixture was then diluted to 5.0 ml and the increase in optical density was measured for an hour at regular intervals in the presence of 1×10^{-3} M ATCh and DTNB. A control without inhibitor showed a constant hydrolysis rate during the period of observation.

In the experiment to study the influence of substrate concentration on the rate of enzymic hydrolysis, the reaction mixture contained 0.05 M Tris-HCl buffer pH 7.5, 1.5 per cent NaCl, 3.3×10^{-4} M DTNB and the desired concentrations of inhibitor and substrate were simultaneously added followed by 0.1 ml of enzyme. The volume of total reaction mixture amounted to 1.5 ml. A reaction mixture without inhibitor served as control while one without both enzyme and inhibitor was kept as blank. The increase in inhibition was measured at one minute intervals until the steady state condition was achieved.

RESULTS AND DISCUSSION

All the parameters of inhibition obtained in the present study are given in Table 1.

The I_{50} value is the inhibitor concentration in moles per litre required to give 50% inhibition when the inhibitor is incubated with enzyme for 30 minutes. The low I_{50} values clearly indicate the high susceptibility of honeybee ChE to carbamate inhibition. This observation was in agreement with the results of EL-AZIZ *et al.* (1969), who also reported an extremely high susceptibility of honey bee to carbamates. The order of inhibition was furadan > 3-OH carbofuran > 3-keto carbofuran. The differences among the I_{50} values obtained for the three inhibitors are not significant. The results gave a clear proof for the high inhibitor potency of furadan towards insect ChE.

Since the affinity constant Ka measures the ability of the enzyme-inhibitor complex to dissociate, the lower the numerical value of this constant, the more effective the binding at the active site of the enzyme. From the data it is apparent that 3-keto carbofuran showed the highest affinity for honey bee ChE, while there was a 12-fold increase in the Ka value with furadan and a 25-fold difference with 3-OH carbofuran. So the order of carbamate inhibitors in the descending pattern of affinity for honey bee ChE was 3-keto carbofuran, furadan and 3-OH carbofuran. The inhibitor constants of 3-keto carbofuran clearly indicate that the selection of a synthetic carbamate for commercial use as an insecticide is largely dependent on its I_{50} value than on its affinity constant.

A general perusal of the carbamylation constants (k_{+2}) indicate that these indices did not differ significantly. The order of

TABLE 1. Inhibition constants for honey bee cholinesterase by various carbamates. Each datum is an average of five replicates.

Sl. No.	Inhibition constant	Inhibitor			Unit
		Furadan	3-OH carbofuran	3-keto carbofuran	
1.	I^{50} value	1.77	1.90	3.35	X 10^{-8}M
2.	Affinity constant, Ka	2.68	5.56	0.22	X 10^{-7}M
3.	Carbamylation constant, k_{2c}	2.80	4.92	6.09	min^{-1}
4.	Bimolecular reaction constant. Ki.	1.40	0.88	27.10	X 10^{7}M^{-1} min^{-1}
5.	Decarbamylation constant, k_3	0.0016	0.0030	0.0030	min^{-1}
6.	Half-life value, $t_{0.5}$	433	231	231	minutes
7.	Average apparent affinity constant, Ka'	2.01	13.30	16.70	X 10^{-7}M.

k_{2c} of honey bee ChE was 3-keto carbofuran > 3-OH carbofuran > furadan. A subsequent study with house sparrow and rat ChE's (data not presented in this paper) also showed only an insignificant difference among k_{2c} values, probably indicating a minor role of the carbamylation step in the overall specificity of each enzyme source to various carbamate inhibitors.

From the data it would be clear that the bimolecular reaction constants, Ki, varied among themselves for all the three carbamate inhibitors. The Ki of honey bee ChE for furadan was almost double than that of its hydroxy analogue while for 3-keto carbofuran, it was about 30 times higher than that for 3-OH carbofuran. The differences in the Ki values would largely be attributable to the variations in the Ka values rather than to the k_{2c} values.

The decarbamylation constants (k_3 values) obtained in the present studies are less than those reported with eel ChE by WILSON *et al.*, (1960). The k_3 value is liable to be affected by various experimental conditions like the source of enzyme, assay, inhibitor, substrate concentration, temperature, pH etc. The results reported in the present investigation may also be taken to suggest the probable difference in the chemical bondage in the honey bee carbamylated enzyme which forms a hindrance for the inhibitor moiety to separate out from the enzyme part. This difference in k_3 value of honey bee enzyme could also be interpreted to indicate the difference at the active site of the enzyme.

The half-life values of the different carbamylated enzymes were calculated using the equation, $t_{0.5} = \frac{0.693}{k_3}$. The carbamylated enzyme of honey bee with furadan took about 7 hours for recovering 50 per cent of its original enzymic activity while those with the two other analogues regained their half-activity after about 4 hours. Hence the higher $t_{0.5}$ value with insect ChE can be considered as an attribute for a good insecticide.

The average apparent affinity constants (Ka') as coined by DAVIES *et al.*, (1970) were obtained from the experiment in which the inhibitor was added simultaneously with the substrate. Ka and Ka' values of furadan for honey bee ChE were almost equal. There was a 2-fold difference in 3-OH carbofuran and a 76-fold difference in the case of 3-keto carbofuran.

In conclusion, it may be pointed out that the results obtained in the present studies

suggest that furadan and its analogues are competitive inhibitors at low concentrations (below 10^{-6} M) and the inhibitions could be predicted by invoking the mechanisms proposed by MAIN & HASTINGS (1966). At higher concentrations of the inhibitors, some other mechanism is also enforced. The deviation observed at higher inhibitor concentrations could perhaps be easily explained on the basis of allosteric inhibition.

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EFFECT OF THE CHEMOSTERILANTS APHOLATE AND METEPA ON THE OVARIES OF THE RED COTTON BUG, *DYSDERCUS CINGULATUS* FABR. (INSECTA, HETEROPTERA, PYRRHOCORIDAE

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Studies on the histology and histochemistry of the ovaries in normal *Dysdercus cingulatus* and effects of the chemosterilants apholate and metepa on the ovaries of this animal are presented. Single injection of 10 μg metepa or 5 μg apholate per newly moulted adult female does not apparently interfere with oocyte growth or vitellogenesis. But the eggs laid after both these treatments do not hatch. Oocyte development is however inhibited after treatment of newly moulted adult females with 12.5 μg metepa or 7.5 μg apholate. Histological and histochemical studies of the ovary after this treatment indicate disintegration of germarium and follicular epithelium as well as reduction in size of oocytes and their resorption. Prefollicular tissue almost completely disappears and multiple oocytes are present in follicles after treatment with apholate. Histochemical studies reveal no yolk granules of protein, carbohydrate or lipid after treatment with both the sterilants. Treatment with apholate by contact method at 0.22 mg/ sq cm of surface of contact, inhibits oocyte growth after ptevitellogenesis, ultimately resulting in irreversible atrophy of the ovarian tubes.

INTRODUCTION

Chemosterilants affect fecundity and fertility of insects (LACHANCE *et al* 1968; CAMPION, 1972), but we know very little about the effects of the sterilants on female reproductive system. Studies on this aspect are limited mainly to flies and mosquitoes (MORGAN, 1967; MORGAN & LABRECQUE, 1962, 1964; RAI, 1964; LANDA & REZABOVA, 1965); our information in this regard extends very little to histological levels even in most of these studies. Detailed studies on the histopathological and histochemical effects of chemosterilants on hemipteran ovaries are fewer. Effects of 6-azauridine have been studied on the ovaries of *Pyrrhocoris apterus* (MASNER, 1971). Severe pathological effects on the ovaries by tepa have been reported in *Dysdercus cingulatus* on the basis of morphological studies SUKUMAR & NAIDU, 1973). The present investigations have been carried out to find out the action of the chemosterilants apholate and metepa on the ovaries of *Dysdercus cingulatus* at histological and histochemical level. Ovaries of the normal animal have also been studied.

MATERIALS AND METHODS

Rearing of animals

The red cotton bug *Dysdercus cingulatus* was reared in the laboratory on soaked cotton seeds kept on wire meshes in plastic basins of 30 cm diameter. The rearing basins were covered with clothing to prevent the animals from escaping. The seeds were changed daily. Mating started when they were two days old. The animals laid eggs when six or seven days old, among cotton seeds, in clutches. These eggs were removed to a Petri-dish while changing the cotton seeds in the morning. The eggs hatched by five days. The first instar nymphs were transferred to cotton seeds and were reared as above in basins. These animals have five nymphal stages and the freshly laid egg took about twenty five days to become adult, under the laboratory conditions.

Animals used

Newly moulted adults were collected from the stock colony. Females could be distinguished by

their larger size and the external genitalia. The newly moulted males and females were transferred to glass chimneys and reared on soaked cotton seeds; the mouth of the chimney was covered with clothing. Thus animals of known age were readily available for the study.

Histological and histochemical techniques

For the study of the normal ovary and vitellogenesis, females were sacrificed at one day interval upto six days post-emergence. The ovaries were dissected out and fixed in BOUIN'S fluid, CARNOY'S fluid, 10% formalin, or ZENKER'S fluid. For histological studies, ovaries fixed in BOUIN'S fluid or ZENKER'S fluid were processed in the routine manner. Paraffin sections were stained in HEIDENHAIN'S iron haematoxylin and eosin.

For histological studies, ovaries were embedded in either paraffin or gelatin. Proteins were demonstrated by MILLON'S reaction (after BAKER) or by mercury bromophenol blue method after BONHAG (PEARSE, 1968), using CARNOY–fixed or formalin–fixed material after embedding in paraffin or gelatin. For carbohydrates, Periodic acid Schiff technique (after MCMANUS) was employed using formalin–fixed gelatin sections or Carnoy–fixed paraffin sections. Lipids were stained using Sudan Black B or formalin–fixed gelatin sections or formalin-fixed paraffin sections. DNA was studied using Feulgen technique, on paraffin sections after fixation in CARNOY'S fluid or formalin fixation. Nucleic acids were also studied by methyl green pyronin Y method (CURNICK) employing formalin–fixed gelatin sections and Carnoy–fixed paraffin sections. Suitable controls were kept for all histochemical procedures.

Chemosterilants

Metepa and apholate were the chemosterilants employed. They were either injected into the haemocoel through pleural region after ether anesthesia by means of a microliter syringe, or the animals were treated by contact method. For injection the solutions were dissolved in distilled water at known concentration. After preliminary trials, 10 μg and 12.5 μg metepa and 5 μg and 7.5 μg apholate were chosen for detailed study. Controls received distilled water injection. For treatment by contact method, sets of PETRI-dishes of radius 5 cm and height 2.5 cm were taken and 50 mg apholate dissolved in 5cc acetone was applied uniformly to each set over all the surface of contact and allowed to dry. This gave a residue of 0.22 mg / sq cm of the surface of contact. Animals kept in acetone-treated dishes served as controls. Both experimental and control animals were kept in the PETRI-dishes for 4 hours. Only newly moulted animals were used for treatment.

Treated as well as control animals along with some normal males were reared on soaked cotton seeds in glass chimneys. Eggs laid by the animals were collected, counted and observed for hatchability studies. Treated insects were also dissected in insect Ringer 2, 3, 4, 5, 6, 12 and 19 days after treatment to note the progressive changes in the ovaries. Ovaries of control insects were dissected out 2, 3, 4, 5 and 6 days after treatment. Histology and histochemistry of the ovaries of these insects were studied as already desceribed.

OBSERVATIONS

Structure of the ovavries and vitellogenesis in the normal animal

The ovaries of *Dysdercus cingulatus* are telotrophic, each with seven ovarioles. The ovariole consists of terminal filament, germarium, vitellarium and pedicel (Fig. 1). In the germarium are the germinal cells at various stages of maturation, trophocytes, trophic core and prefollicular tissue. As each oocyte of the germarium enlarges and enters the vitellarium, prefollicular tissue which is at first multilayered, gets arranged around the oocyte in the form of follicular epithelium. This becomes ultimately one layer thick. During the previtellogenic stage which extends upto two days after emergence this differntiation of follicular epithelium takes place. The follicle cells which are at first small and mononucleate, subsequenly enlarge and become binucleate. Vitellogenesis begins on the third day and continues on the fourth and fifth days. Concomitant with the progress of vitellogenesis the abdomen swells to accommodate the enlarging oocytes.

The cytoplasm of the trophocytes, young oocytes, follicular epithelium as well as the trophic core and nutritive cords of the previtellogenic ovary are stainable rather homogeneously with techniques for proteins and RNA but no granules of these materials are visible now. The trophocytes apparently

contribute DNA, large quantities of RNA and proteins to the developing oocytes. Masses of DNA are visible in the trophocyte region, but the trophic core is not Feulgen positive. DNA appears to break down before reaching the trophic core. Small granules which are rich in both carbohydrates and proteins appear in the peripheral ooplasm of the basal oocytes on the third day. These enlarge in size and number forming the large yolk granules made up of protein-carbohydrate complex (Fig. 2). These subsequently fill the oocyte. When vitellogenesis is most active, spaces develop among follicle cells through which material can enter the oocytes from blood (Fig. 3). Smaller, lipid yolk granules which appear subsequently, are distributed amog the larger protein-corbohydrate yolk granules (Fig. 4). Vitellogenesis is complete by the end of the fifth day and chorion formation ensues subsequently. Completed, mature eggs are deposited on the sixth or seventh day.

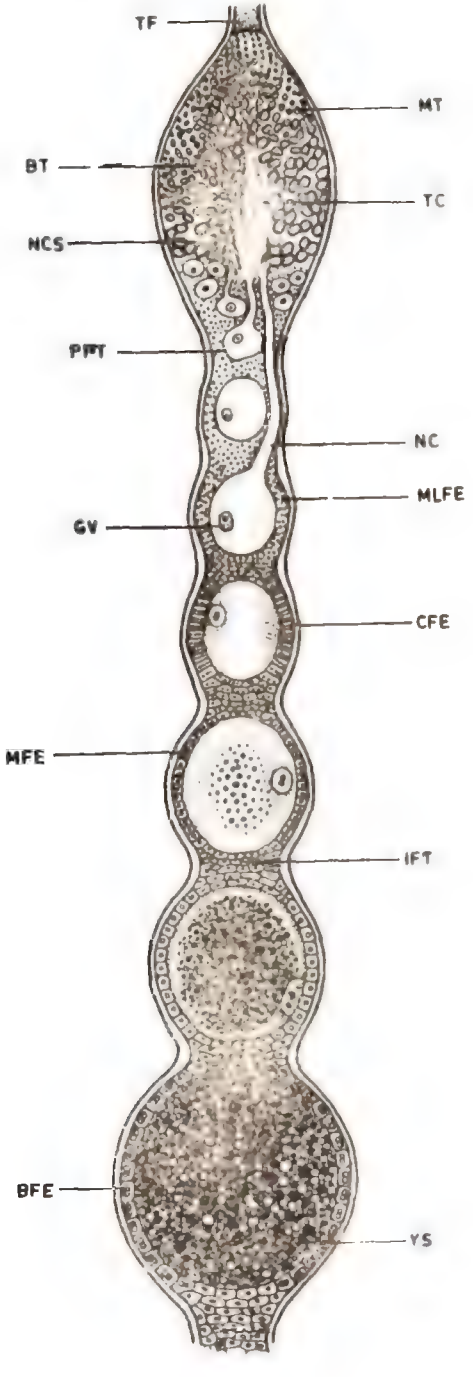


Fig. 1

Effect of metepa

Animals treated with 10 μg metepa lay sterile eggs. This dosage does not interfere with the development of the ovary or with fecundity. Mating is normal in treated insects. Insects which receive 12.5 μg metepa appear normal on the day of the injection and on the next day. However, on the third day about 50% mortality is recorded among them. The remaining animals mate freely. Abdomen does not swell even after 12 days indicating absence of egg development. This is substantiated histologically. Infecundity is complete. Insects have been kept upto nineteen days to see whether the trend is reversed and eggs start developing again. They have been found to develop no eggs. Thus 12.5 μg metepa inhibits growth of the ovary and vitellogenesis completely.

Detailed studies on the histological changes in the ovaries after treatment with 12.5 μg metepa reveal that ovariole size as well as oocyte number are reduced in the treated insects. Visible histological changes in the

EXPLANATION OF FIGURES

Fig. 1. Diagram representing the longitudinal section of an ovariole of 3–4 day old *Dysdercus cingulatus*. BFE–binucleate follicular cell; BT–binucleate trophocyte; CFE–columnar follicle cell ; GV–germinal vesicle ; IFT–interfollicular tissue ; MFE–mononucleate follicle cell; MLFE - multilayered follicle epithelium ; MT–mononucleate trophocyte ; NC–nutritive cord ; NCS–nuclear clusters of trophocytes ; PFT–prefollicular tissue ; TC–trophic core ; TF–terminal filament; YS– yolk spheres.

Fig. 2. Section of a mature oocyte showing carbohydrate yolk granules (CARNOY'S fluid ; PAS technique).

Fig. 3. Section of a mature oocyte showing binucleate follicle cells (solid arrows). The thin connection between follicle cells (broken arrows) disappears except in certain regions.

Fig. 4. Section of a mature oocyte showing distribution of the lipid yolk spheres (dark) around the protein-carbohydrate yolk granules (unstained). (Formalin, Sudan Black B, cryostat sections).

Figs. 5 & 6. Section of the germarium of animals three days after injection of 12.5 μg metepa (Fig. 6) showing disintegration of the posterior region of the germarium (dark masses), and section of its control (Fig. 5) showing less intense trophocyte disintegration.

Fig. 7. Section of the germarium and part of the vitellarium of an animal seven days after injection of 12.5 μg of metepa showing the trophic tissue almost completely dis integrated (dark masses). Arrow shows hyperplastic follicle cells.

Fig. 8. Section of a part of the vitellarium of an ovariole four days after injection of 12.5 μg of metepa showing resorbing oocyte (RO) and the hyperplastic follicular epithelium (FE).

Fig. 9. Section of the ovariole of an animal nineteen days after injection of 12.5 μg metepa. Note the disintegrated content (G) at the basal region of the germarium. Vitellarium is almost empty.

Fig. 10. Section of the ovary of an animal two days after injection of 7.5 μg apholate. Note the disintegration of trophocytes (DT), absence of prefollicular tissue and poorly developed follicular epithelium.

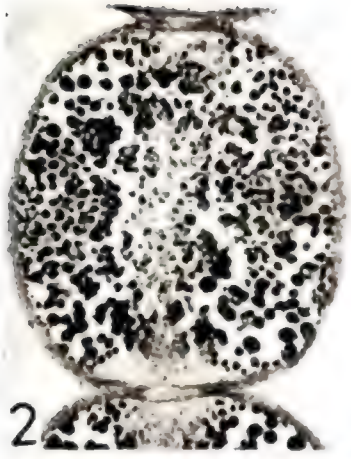
Fig. 11. Section of the germarium of ovaries of an animal four days after injection of 7.5 μg apholate, showing dark staining granules in the oocytes and the disintegrated trophocytes.

Fig. 12. Section of the vitellarium of an animal four days after injection of 7.5 μg apholate, showing twin oocytes in a follicle (arrows) and poorly developed follicular epithelium.

Fig. 13. Section of the vitellarium showing resorption of the oocyte (dark masses) eight days after injection of 7.5 μg apholate.

Fig. 14. Section of the ovary of an animal nineteen days after apholate treatment by contact method, showing atrophied ovarian tube and invading follicle cells (arrows).

(All figures from 2–14, unless otherwise stated, are from preparations made from tissues fixed in BOUIN'S fluid and stained in HEIDENHAIN'S iron haematoxylin and eosin).



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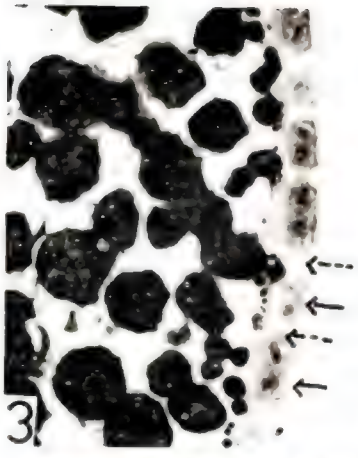

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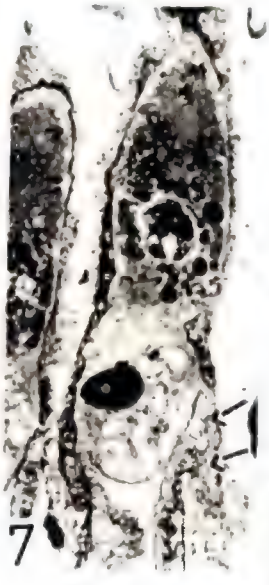

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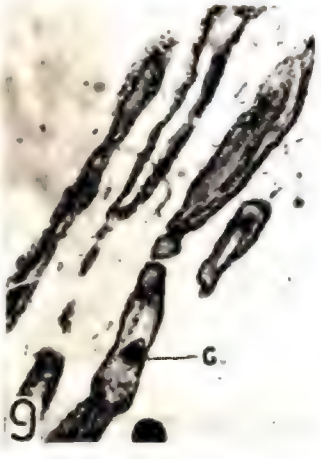

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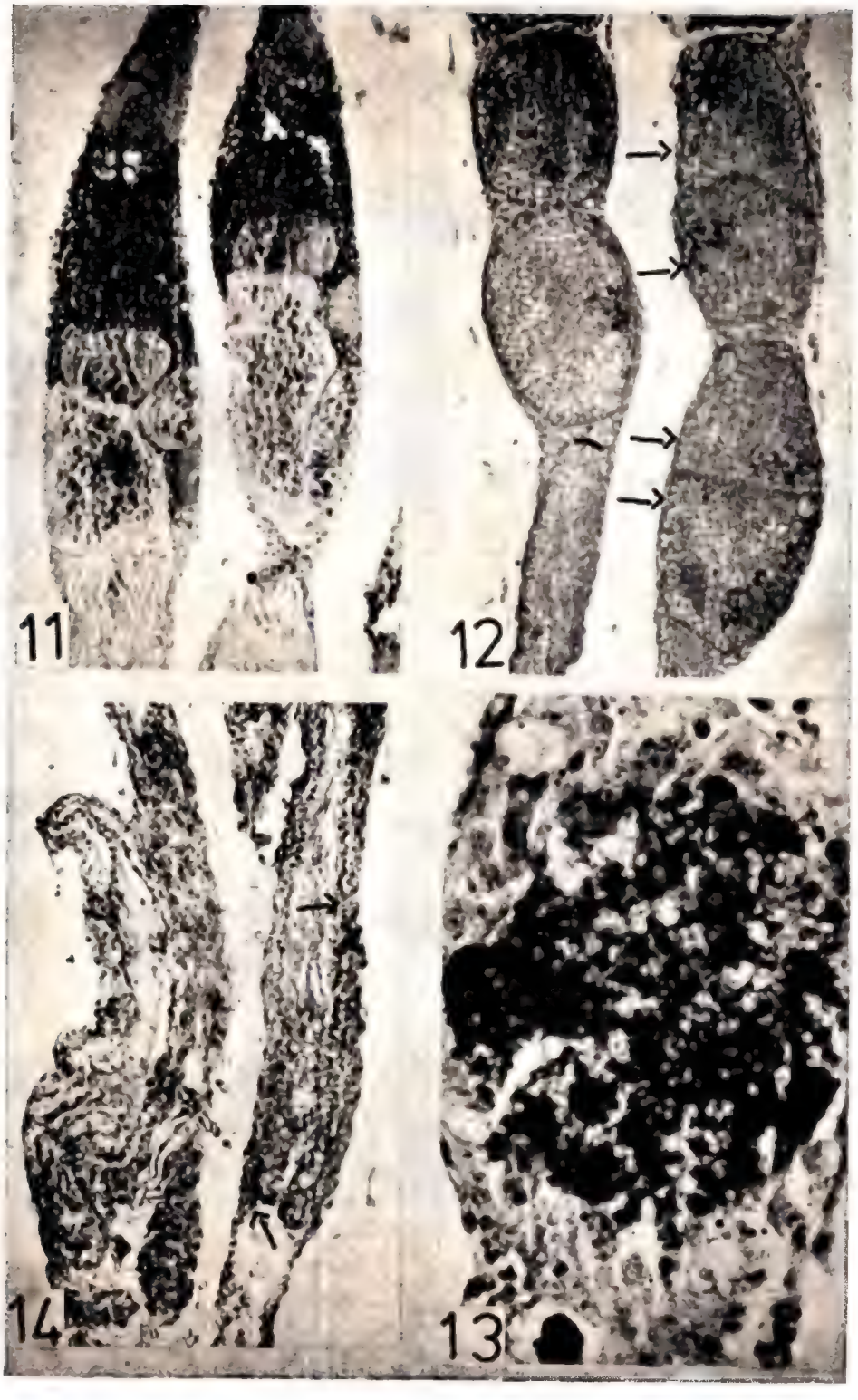
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ovary start two days after injection. Intense disintegration of the trophocytes starts from the posterior zone of the germarium and progresses forwards (Figs. 5&6). Ovariole at this stage measures 1.7 mm in length as compared to control ovariole which is 4 mm in length. There are now four to six oocytes in the vitellarium which are all at previtellogenic stage in the treated ovariole whereas in the corresponding controls the basal oocytes start deposition of yolk granules.

Disintegration of chromatin material of the trophocytes, prefollicular tissue and germ cells increases steadily, forming clumps. The cytoplasm also degenerates subsequently contributing to the clumping masses, which ultimately fill the entire germarium. These clumps unite to form large masses which develop among them vacuoles. Six to seven days after treatment the germarium is almost full of disintegrated tissue (Fig. 7).

Of the 4–6 oocytes in the vitellarium none show signs of any yolk deposition when examined on the fourth day. The treatment results in resorption of the oocytes even before yolk formation, and it starts most frequently from the anterior end of the vitellarium but occasionally from the posterior end also. Resorption starts on the second day or on the early third day after treatment. At first the follicular epithelium appears quite normal. Some light–staining patches appear in the homogeneous ooplasm which later develops vacuoles. Gradually vacuolation of the cytoplasm increases, resulting in masses of dark staining bodies. During this time the follicular epithelium shows considerable changes. In some ovaries, follicular epithelium appears multilayered and results in hyperplasia (Fig. 8). When compared to the follicle cells of the control which measure about 17 μ x 15μ, those of treated insects are very small measuring only 7μ x 3μ. They do not differentiate beyond this stage. Later they become irregular in shape, pycnotic and gradually appear to invade the oocytes. The shape of the oocyte becomes distorted and the oocytes grow smaller as a result of gradual resorption. By six or seven days after treatment almost all the oocytes are resorbed and their place is occupied by the follicle cells which now increase in size and fill the entire "ooplasm". The mass of follicle cells and the relics of the germarium get shrunk, ultimately leaving the ovariole as an almost empty tube (Fig. 9). No sign of development of a second batch of eggs is indicated even in animals kept under observation for nineteen days.

In the resorbing oocytes in animals six days after treatment, certain regions of the cytoplasm show abundant proteins and the vacuolated region contains very little of it. The clumped material of the germarium is lightly stainable with PAS. As vitellogenesis does not take place in the treated ovary the corresponding histochemical reaction is absent. Most of the clumps of degenerated material in the treated ovary consist of either DNA or RNA. Material taking up both methyl green and pyronin and hence containing both DNA and RNA are also present among them. The ovary of the control animal does not differ from that of the normal animal either histologically or histochemically.

Effect of apholate

Females treated with 5 μg of apholate show a mortality rate of about 25%. They deposit sterile eggs. Rather high mortality of about 50% is observed among insects treated with 7.5μg of apholate. These insects mate less frequently and swelling of the abdomen is not noted. Dissection of these insects reveals drastic influence on ovaries. Apholate-treated ovary presents a histological pattern of degeneration different from that of the metepa-treated ovary.

Changes in the ovary are noticeable even from the beginning of the second day (Fig. 10). There are fewer oocytes in the vitellarium and as a result the whole ovary is reduced in size. Disintegration of the germarium starts from the posterior region and proceeds anteriorly. Four days after treatment the whole germarium is filled with irregular clumps of chromatin material (Fig. 11). Almost complete elimination of perfollicular tissue is a striking feature of the ovary as early as two days after treatment (Fig. 10). As a result the follicular epithelium is almost reduced to a thin membrane without any distinct cells. More than one oocyte very often occur in a single follicle (Fig. 12). The linear arrangement of follicles in the vitellarium is also disturbed. Oocytes with apparently two nuclei are sometimes found in the germarium. Yolk deposition does not take place in these oocytes and these are never oviposited. They later disintegrate and are resorbed. Vacuoles appear in the homogeneous cytoplasm of oocytes three days after treatment along with granules which stain dark by iron haematoxylin. In oocytes four or five days after treatment, these dark staining granules increase in size, later forming dark masses filling the entire oocyte (Fig. 13). They differ from the yolk granules in that they first appear in the oocytes which are either in the germarium or are near it. They are also irregular in shape. These bodies later get resorbed and their place is occupied by the invading follicle cells.

Protein granules indicating resorption are observed in the cytoplasm and nuclei of oocytes, follicular epithelium and trophocytes of the treated ovary as early as one day after treatment, unlike the controls. In animals four days after treatment the degenerating material in the germarium and in the oocyte is rich in protein, but the controls contain no such granules. These however are not comparable to yolk granules. As vitellogenesis does not take place in the treated ovary, the protein-carbohydrate yolk granules and lipid yolk granules are entirely absent in them. The disintegrated mass of the trophocytes and what remains of the follicular epithelium and oocytes, contain DNA and RNA. These clumps fill the entire germarium as well as the vitellarium of the ovaries six days after treatment.

Effect of apholate treatment by contact method

Newly moulted females exposed to apholate residue fail to develop eggs. Oocyte growth proceeds upto the end of the previtellogenic period. Sometimes vitellogenesis starts but later those oocytes also get resorbed. The ovary nineteen days after treatment show atrophied ovarian tube, the interior of which is filled with the invading follicle cells (Fig. 14). The shrunken, disintegrated germarium consists only of the fragments of cytoplasm and disintegrated chromatin material. Control insects exposed to acetone treated surface are comparable to normal animals.

DISCUSSION

The structure of the ovaries and vitellogenesis in *Dysdercus cingulatus* are essentially comparable to that in *Dysdercus fasciatus* worked out in detail by BRUNT (1971). Yolk deposition in *Dysdercus cingulatus* starts on the third day after adult emergence, as already reported (JALAJA & PRABHU, 1971).

Apholate and metepa administered in various doses produce changes which range from an almost complete necrosis of the ovary to almost normal ovary resulting in the production of apparently normal but nonviable eggs. The severity and changes produced by the chemosterilants are dependent on dosage. The infertility of the apparently normal eggs produced as a result of

treatment of females with lower doses may be due to enzyme inhibition (MENDOZA & PETERS, 1968; TURNER & MAHESWARY, 1969), or due to induction of dominant lethal mutations as suggested by LACHANCE & CHRISTAL, (1963) in other insects.

The two chemosterilants apholate and metepa act differently on the ovarian tissue though the final result is the same. When higher doses are employed reduction in the number of oocytes as well as decrease in the size of the ovary are the common effects. The trophocytes of the germarium are the first to be affected by the sterilants. At the time of the treatment the vitellarium already contains three to four oocytes. Production of more oocytes is inhibited by the sterilants. This is due to disintegration of the posterior zone of the germarium where oocytes are differentiated. The disintegration of the germarium leads also to restriction of the supply of trophic material such as ribonucleoprotein and DNA necessary for the activation of young oocytes in the vitellarium, restricting their growth during the previtellogenic period. The observations on the house flies also indicate that damage to follicle cells and nurse cells of the egg chamber as well as destruction of the germarium are responsible for infecundity (LANDA & REZABOVA, 1965). Ovarian degeneration is also observed in *Drosophila* (CANTWELL & HENNEBERRY, 1963) after treatment with chemosterilants. In the telotrophic ovary of beetles disintegration of the upper part of the germarium and suppression of the division of trophocytes are noted following application of tepa (ONDRACEK & MATOLIN, 1971). Atrophy of prefollcular and follicular tissues and the occurrence of multiple oocytes within a single follicle without any linear arrangement of oocytes in the ovarioles are the striking characteristics of apholate treated ovary observed during the present study. In *Pyrrhocoris* this phenomenon has been described after treatment with 6-azauridine in which it has been suggested that the twin oocytes are formed as a result of disturbance of the division mechanism by the chemosterilant (MASNER, 1971). It may be due to oocyte fusion or due to more than one oocytes entering the same follicular chamber.

There is a possibility that yolk deposition does not start in the oocytes of the treated insects due to failure of follicle cells to differentiate properly. Synthesis of yolk in many insects does not start until follicular epithelium differentiates properly (ANDERSON, 1971). The sensitivity of the follicular epithelium to the sterilants observed during the present study is especially significant in the light of the findings that follicle cells play an important role in incorporating yolk into the oocytes (ANDERSON & TELFER, 1969). Chemosterilants also influence the neuroendocrine mechanism in insects (MASNER & MACHA, 1968; TAN, 1974; JALAJA & PRABHU, 1976). Median neurosecretory cells and corpus allatum are known to control vitellogenesis in this animal (JALAJA, 1974, 1975; JALAJA *et al.*, 1973). So the effects of the sterilants on the neuroendocrines might also play their role in affecting vitellogenesis in the treated ovary. Resorption in insects occurs after treatments with chemosterilants (SMITTLE *et al.*, 1966: BULYGINSKAYA *et al.*, 1967: CAMPION, 1968; KERNS & NAIR, 1972; SUKUMAR & NAIDU, 1973). In *Schistocerca* (KERNS & NAIR, 1972) the lower concentration of haemolymph proteins in the tepa-treated insects is one of the factors that contribute to the resorption of oocytes. However, in metepa treated cockroach *Periplaneta* haemolymph protein concentration does not fall, though there is a change in electrophoretic pattern of the blood proteins (PRABHU & NAYAR, 1972). It appears that a number of these factors might play their role in bringing about inhibition of vitellogenesis in *Dysdercus* after treatment with the chemosterilants.

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RESIDUES OF ALDRIN IN POTATO

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Application of aldrin 5% dust @ 25 kg ha to soil before planting potatoes for the control of soil pests resulted in residues of aldrin+dieldrin to the extent of 0.8 to 1 ppm in the tubers at harvest. Separate analysis of peel and pulp revealed the presence of 1.75 ppm and 0.21 ppm residues, respectively. Both aldrin and dieldrin were found present in the peel whereas only dieldrin was detected in the pulp. Washing and cooking did not have significant impact on the removal of residues. The total residues (aldrin+dieldrin) on the whole potatoes, the peel or the pulp were always more than the tolerance limit set by FAO/WHO (0.1 ppm). It is, therefore, concluded that potatoes grown in aldrin-treated soil are hazardous to consume.

INTRODUCTION

Application of 5% aldrin dust to soil before planting potatoes @ 25 kg/ha has been recommended for the control of cutworms, crickets, white grubs and termites (REDDY, 1968; BANERJEE, 1970) and is being widely followed. In certain cases still higher dosage of 3.36 kg a.i./ha, half applied at the sowing time and half at the earthing time, has also been recommended (ABRAHAM *et al.*, 1972). Very little information is available on its residues in tubers. SINGH & KALRA (1971) studied the residues of aldrin using emulsion @ 2 kg a.i./ha at the time of earthing up. However, such a high dose is rarely used and also this practice is uncommon. Hence it was considered important to study the level of residues following the aforesaid normal recommendation.

MATERIALS AND METHODS

The potato crop was grown on the farm of Indian Agricultural Research Institute in a small area of 1/5 hectare. Aldrin 5% dust at 25 kg/ha was mixed in the soil before planting the tubers. The residues were determined in the harvested crop separately in unwashed and washed potatoes and in peel and pulp before and after cooking. For washing, the potatoes were taken in a tray and washed under tap water for 2 minutes, gently rubbing with hands. The potatoes were cooked in a metallic pan in sufficient water till they became soft. For each estimation a bulk sample of 2 kg potatoes of various sizes taken at random was drawn. Depending upon the type of estimation, the tubers or pulp or peel, were finely dropped and mixed thoroughly. Out of this a representative sample of 50 g was taken and exatracted with hexane and acetore (80: 20). The extracts were cleaned up with hyflo-supercel plus anhydrous sodium sulphate plus activated charcoal (5 : 5 : 1) by column chromatography. The residues were determined by bioassay as described by ATTRI & RATTAN LAL (1972) and also by GLC using 63Ni electron capture detector. The GLC parameters were : detector temperature: 250°C, column temp 200°C, inlet post temp 225°C, nitrogen carrier gas flow rate 80 ml / min, attenuation 0.08, column 180 cm long packed with 3% OV 1 on chromosorb W, 80–100 mesh.

RESULTS AND DISCUSSION

Examination of the average residues determined by bioassay and GLC reveals that estimates by the former are 2–3 times higher than those by the latter method. Obviously this could be due to the presence of dieldrin, a more toxic metabolite of aldrin. The presence of considerable residues of dieldrin in the samples has been amply demonstrated by GLC. The bioassay here, therefore, reflects only the presence of more

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TABLE 1. Residues of aldrin in potato estimated by bioassay and GLC

Sample	Replication	Bioassay	Residues (ppm) GLC		
			Aldrin	Dieldrin	Aldrin plus Dieldrin
Whole potatoes unwashed	R1	1.96	0.35	0.59	0.94
	R2	2.35	0.37	0.64	1.01
	R3	2.50	2.24	0.35	0.59
	Average	**2.27**	**0.32**	**0.53**	**0.85**
Whole potatoes washed	R1	2.85	0.31	0.64	0.95
	R2	2.35	0.21	0.38	0.59
	R3	1.17	0.31	0.54	0.85
	Average	**2.16**	**0.28**	**0.52**	**0.80**
Peel uncooked	R1	4.18	0.65	0.85	1.50
	R2	3.06	0.63	0.76	1.39
	R3	5.25	0.97	1.38	2.35
	Average	**4.16**	**0.75**	**1.00**	**1.75**
Pulp uncooked	R1	0.88	ND	0.24	0.24
	R2	1.19	ND	0.19	0.19
	R3	0.84	ND	01.19	0.19
	Average	**0.94**	**ND**	**0.21**	**0.21**
Peel of cooked potatoes	R1	5.64	0.86	1.32	2.18
	R2	5.40	0.79	1.28	2.07
	R3	3.15	—	—	—
	Average	**4.73**	**0.82**	**1.30**	**2.12**
Pulp of cooked potatoes	R1	1.14	ND	0.33	0.33
	R2	1.02	ND	0.35	0.35
	R3	0.98	ND	0.19	0.19
	Average	**1.04**	**ND**	**0.29**	**0.29**

ND — Not detectable.

toxic metabolite(s) rather than the exact magnitude of residues of the original compound and its metabolite(s).

Considering the combined residues of aldrin and dieldrin, it is observed in Table 1 that whole potatoes with peel contain residues ranging from 0.6 to 1 ppm with an average of 0.85 ppm when unwashed and 0.8 ppm when washed. In other words, this further means that washing has no effect in removing the residues from the whole tubers. FAO/WHO (1975) recommended tolerance limit for aldrin+dieldrin to be 0.1 ppm on potatoes, which has been accepted for adoption by the Central Committee on Food Standards, Directorate General of Health Services, New Delhi. In this experiment the combined levels of aldrin and dieldrin as mentioned above were far more than the tolerance limit set by FAO/WHO. Therefore, it is concluded that potatoes grown in aldrin-treated soil are hazardous to consume. Similar conclusions were drawn by SINGH & KALRA (1971) who observed that application of aldrin @ 2 kg a.i./ha (which was though much higher than that applied in the present experiment) resulted in aldrin residues ranging from 0.2 to 0.5 ppm and dieldrin residues from 0.6 to 1.5 ppm. Our findings are, however, contrary to those by LICHTENSTEIN & SCHULZ (1965) who observed that potato, radish and carrot grown in soil treated with aldrin @ 1 lb/acre either contained no residues or residues at the concentration of 0.03 to

0.05 ppm. In addition, our results also do not agree with those summarised by FAO/WHO (1971) where most of the data presented by Shell Research Ltd. show the residues to be less than 0.15 ppm. The soil type and climatic factors might be responsible for variation in the results. This further suggests the need for extensive research under Indian conditions even though the data collected over the western world is available at hand.

When the residues were determined separately in peel and pulp, it was interesting to note that the peel contained 8–9 times more residues than the pulp on weight to weight basis. Peel and pulp (uncooked) contained on average 1.75 ppm and 0.21 ppm residues respectively. The bioassay results also show a similar trend so that the residues in the peel were about 5 times higher than those in the pulp. This proportion of the residues in the peel and pulp appears to be maintained in the corresponding cooked sample. Taking into consideration 0.80 ppm residues in whole potato and 0.21 in pulp (Table 1), it may be said that about 80% of the residues remain confind to the peel and only 20% enter the pulp.

Cooking of the samples resulted in slight increase in the average residues both in the peel and in the pulp as is evident from bioassay and GLC estimates. A possible explanation can be that the operation of cooking unlocked the bound residues in the material which became readily extractable.

The analytical data do not show the presence of aldrin in the pulp. Whether aldrin is immediately converted into dieldrin in the pulp or there is selective translocation of dieldrin is immediately converted into the pulp is not known, Both aldrin and dieldrin occur in the peel. Dieldrin was present always in greater amounts than aldrin, the ratio being 1:1.3 to 1:1.5. Cooking did not significantly change this ratio. These results confirm the findings of SINGH & KALRA (1971) who found that in whole potatoes (since the peel was also analysed alongwith) the level of aldrin ranged between 0.2 to 0.5 ppm whereas that of dieldrin between 0.6 to 10.5 ppm.

The water in which the potatoes were boiled was also analysed and was found to contain only dieldrin to the extent of 0.02 to 05 ppm.

GLC measurements on cleaned up extracts from all the samples from the treated plots showed presence of about 4–5 units high unidentified peak having a retention time of 36 seconds. A similar peak was observed by SINGH & KALRA (1971) also. LICHTENSTEIN *et al.*, (1970) and IVIE & CASIDA (1971) observed the formation of photo-aldrin and photo-dieldrin in soil and plants as a result of exposure to sunlight. Number of other metabolites have also been found to occur in plants (KORTE, 1970). The additional peak as observed during the course of present investigation may be, therefore, due to one of such metabolites of aldrin and dieldrin.

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STUDIES ON THE APHIDS (HOMOPTERA: APHIDIDAE) FROM EASTERN INDIA XXXIV. TWO NEW GENERA, THREE NEW SUBGENERA, ONE NEW SPECIES AND SOME NEW RECORDS FROM NORTH EAST INDIA

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Two genera viz., *Neohyalomyzus* and *Scleromyzus*, three new subgenera viz., *Anacyrthosiphon*, *Neomacrosiphum* and *Paraneomyzus*, one new species viz., *Xenomyzus scabripes* are described Further the genus *Xenomyzus* and the species *Dactynotus (Uromelan) gobonis* are reported for the first time from India.

Re-examination of the aphid fauna collected from Eastern India has necessitated erection of two new genera, viz., *Neohyalomyzus* with *Hyalomyzus raoi* HILLE RIS LAMBERS as type, *Scleromyzus* with *Myzus corylopsis* as type; three new subgenera, viz., *Anacyrthosiphon* with *Neoacyrthosiphon* (*Pseudoacyrthosiphon*) *takahashii*, as type, *Neomacrosiphum* with *Sitobion pseudoluteum* as type, *Paraneomyzus* with *Aulacorthum* (*Neomyzus*) *dicenirae* as type, and one new species, viz., *Aenomyzus scabripes*. Besides, the genus *xenomyzus* and the species *Dactynotus* (*Utomelan*) *gobonis* are reported for the first time from India. Further, the apterae viviparae of *Hyalomyzus* ?*sensoriatus* reported by GHOSH *et al.* (1971) has been described.

Anacyrthosiphon subgen. nov.

Anacyrthosiphin is described here as a new subgenus under *Pseudoacyrthosiphon Pseudoacyrthosiphon* was described by GHOSH & RAYCHAUDHURI (1969) as a subgenus of *Neoacyrthosiphon* TAO with *holstii* TAKAHASHI as type species, because of presence of spinules on ultimate rostral segment and on hind tibiae of nymphs. Later another species viz, *takahashii* GHOSH was found to possess the above mentioned characters as in *holstii*. So it appears that in nature a population exists which has spinulosity on ultimate rostral segment as well as on hind tibiae of nymphs while some other population does not have them and it is for this reason that *Pseudoacyrthosiphon* has been given full generic status for accomodating species having the above named characters and *Neoacyrthosiphon* is reserved for those species which lack them. GHOSH & RAYCHAUDHURI (1969) wrongly described first tarsal chaetotaxy of *holstii* as 3, 3, 3 which should be as 4, 4, 4 as revealed by re-examination of the same material. In *takahashii* first tarsal is 4, 4, 4. Thus it appears that first tarsal chaetotaxy is variable in *Pseudoacyrthosiphon*. Here 4, 4, 4 hairs on first tarsal segment is considered as character for *Pseudoacyrthosiphon* s. s. and a new subgenus *Anacyrthosiphon* is erectep for species having first tarsal chaetotaxy as 3, 3, 3.

Type species : *Neoacyrihosiphon* (*Pseudoacyrthosiphon*) *takahashii* GHOSH

Dactynotus (*Uromelon*) *gobonis*(MATSUMURA)

Many apterous viviparous $\frac{0}{+}$ $\frac{0}{+}$, India: West Bengal: Darjeeling, from *Carthamus* sp.

Hyalomyzus ? *sensoriatus* (MASON)

Apterous viviparous female : Body about 1.54 mm long with 0.76 mm as the

All the material are in the collection of the Aphid Research Unit, Entomology Laboratory, Department of Zoology, University of Calcutta.

maximum width. Head spinulose on both surfaces except the disc, which is smooth, without median frontal prominence; lateral frontal tubercles well developed, scabrous with the inner margin somewhat converging; anteriormost dorsal cephalic hairs long and fine while those on posterior part short and blunt. Antennae 6-segmented, shorter than to nearly as long as body; basal 2 segments scabrous and the inner margin of segment I bulged; flagellum imbricated but sometimes the outer margin of segment III smooth; flagellar hairs short with blunt to slightly acuminate apices; secondary rhinaria absent; primary rhinaria non-ciliated and not protuberant; p. t. distinctly longer than the base of the segment VI. Ultimate rostral segment nearly as long as h. t. 2 and normally wilh 2 secondary hairs. Thoracic and abdominal tergites pale, rugose, sometimes rugosities appearing as transversely elongated cells and with the post siphuncular segments bearing transverse rows of spinules. Dorsal abdominal hairs short and blunt but on tergite 8 rather long. Siphunculus distinctly swollen on distal 0.50 portion, strongly imbricated, much longer than cauda, apically constricted just before the thick distinct apical flange. Cauda short with a blunt apex and slightly constricted at middle, with 2 pairs of hairs. Abdominal venter with transverse spinulose striae; ventral hairs longer than the anterior dorsal hairs with incrassate apices. Femora and tibiae smooth; 2nd tarsal segment with normal imbrications F. T. C. 3, 3, 3. Nymphs with hind tibiae spinulose.

Measurements of one specimen in mm: Length of body 1.54, width 0.76: antenna 1.08, segments III: IV: V: VI 0.27: 0.16: 0.15: (0.11+0.25); u. r. s. 0.07; h. t. 2 0.07; siphunculus 0.31; cauda 0.06.

Material

2 apterous viviparous ♀ ♀ and 3 nymphs, India: West Bengal: Darjeeling, 13. iv. 1969 from an unidentified plant. coll. M. R. GHOSH.

Remark:

GHOSH *et. al.*, (1971) for the first time just reported the species by apterae viviparae from West Bengal. Their argument for naming the species as *sensoriatus* (MASON) was that the processus terminalis was twice as long as the base of antennal segment VI as is found in *sensoriatus*. That in the described alatae of *sensoriatus* processus terminalis is twice as long as base of segment VI has been quoted by RICHARDS (1958) as the only point of difference from the genotype of *Hyalomyzus*. In view of having only 2 apterae and in not having access to *eryobotriae* of which both apterae and alatae are known and to alatae of *sensoriatus*, the Indian material are described in detail as that of *sensoriatus* with a (?) mark before the specific name.

Neohyalomyzus gen. nov.

Head distinctly spinulose along the posterior margin both dorsally and ventrally and locally spinulose near the base of the antennae leaving the frons and median area of the dorsum smooth (Fig. 1 A), head is also spinulose anteriorly; laternal frontal tubercles well developed, scabrous with inner apices protracted inwards; median frontal prominence well developed; dorsal cephalic hairs stout, fairly long with incrassate apices and placed on high sockets. Antennae 6-segmented, shorter to slightly longer than body; segment I slightly shorter than wide, locally scabrous with the inner surface slightly bulged inwards, segment II much shorter than segment I, scabrous, imbrications on segment III sparse and restricted only near the base, rest of the flagellum in apterae smooth; apterae without any secondary rhinaria, alatae with secondary rhinaria on segments III and IV, flagellar hairs short, thick with incrassate apices; p. t. about 3.0–4.0 × the base of segment VI; primary

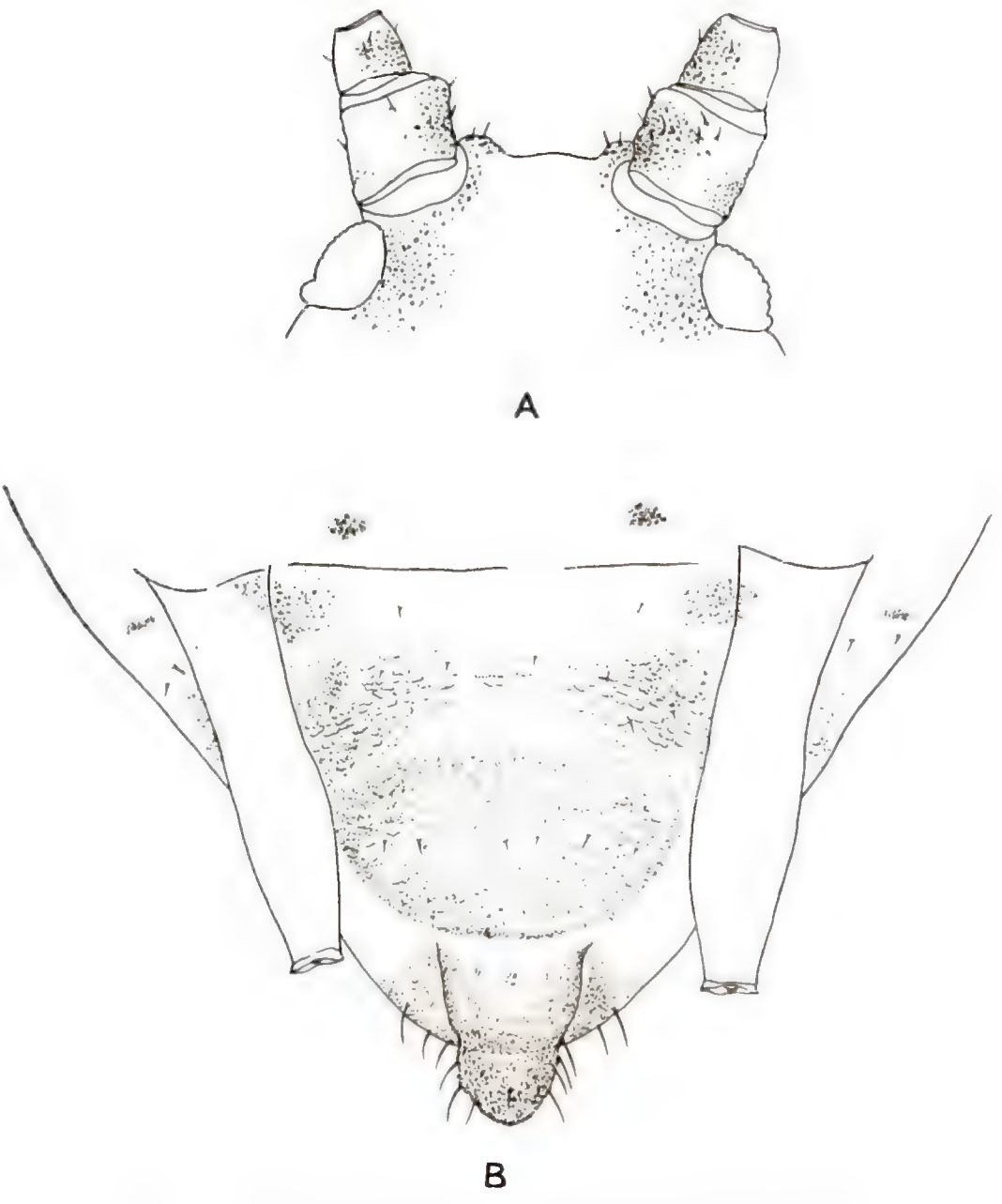

Fig. 1. *Neohyalomyzus raoi* (HILLE RIS LAMBERS), Apterous viviparous female. A. Head. B. Posterior portion of abdomen.

rhinaria non protuberant and ciliated. Rostrum extends upto hind coxae; u. r. s. normal, about 1.12–1.30 × h. t. 2 and bears a pair of secondary hairs. Thoracic dorsum in apterae wrinkled and venter locally spinulose. Mid thoracic furca with a short stalk. Abdominal dorsum in apterae wrinkled (Fig. 1 B) dusky with diffused brown paired muscle plates pleurally, in alatae pigmented, dorsal hairs short with increassate apices and placed on high sockets. Siphunculi more or less cylindrical on basal 0.50 portion and it is followed by a slightly swollen portion which gradually narrows distally, sparsely spinulose, with the apical flange indistinctly or distinctly developed and with a few rows of transverse striae in the preapical circumcised portion, about 0.16–0.22 × the body. Cauda thick, blunt, with the basal 0.50 portion rather swollen. about 0.40–0.55 × the siphunculi and bears 6–8 hairs. Coxae spinulose, rest of the leg rather smooth except the second tarsal segment which are with normal imbrications; hairs on legs short and blunt, F. T. C. 3, 3, 3 wing venation normal. Nymphs with hind tibiae spinulose.

Type Species:

Hyalomyzus raoi HILLE RIS LAMBERS 1973.

Remark:

The new genus is erected with *Hyalomyzus raoi*. HILLE RIS LAMBERS (1973) described the *raoi* under *Hyalomyzus* with reservations since his specimens had median frontal prominence and pre-apical incision on the siphunculi as in *Hyperomyzus* and *Nasonovia* along with characters of *Hyalomyzus*. Examination of a sizeable number of specimens has revealed the presence of inwardly directed apex of scabrous lateral frontal tubercles in both apterae and alatae and a median frontal prominence in apterae; nearly smooth dorsal of head in apterae, rugose body in apterae; pigmented dorsum of abdomen in alatae; clavate siphunculi with a pre apical circumcision in apterae and alatae, hairs on 8th abdominal tergite with acute apices and presence of 3 hairs on first tarsal segments.

This combination of characters justifies the erection of the new genus *Neohyalomyzus* with *hyalomyzus raoi* HILLE RIS LAMBERS as the type.

Distribution: India

Neomacrosiphum subgen nov.

HILLE RIS LAMBERS (1939) while discussing in detail about the genus *Macrosipbum* did not mention about the spinulosity on the head and first tarsal segments with hairs for the species available to him then. In India beside typical *Macrosiphum* species some other species closely similar to typical *Macrosiphum* species have been found, some of these have first trasal segments with 4 pairs and some have spinulose either on both surfaces of head or on only one surface. TAKAHASHI (1961) erected the genus *Unisitobion* for *Macrosiphum*–like species possessing spinulosity on the venter of head, pigmented abdominal dorsum and high antennal sockets but such species having first tarsal segments with 3 hairs. Since in India the *Macrosiphum*–like species viz, *microspinulosum* DAVID, RRJASINGH & NARAYANAN, *pseudoluteum* GHOSH, *pseudogeranii* CHAKRABARTI & RAYCHAUDHURI have 4, 4, 4 as the first trasal chaetotaxy; a new subgenus *Neomacrosiphum is* erected under *Macrosiphum* to accomodate these.

Type species:

Sitobion pseudoluteum GHOSH, 1969

Distribution: India

Paraneomyzus sbgen nov.

All the species known under the genus *Neomyzus* have first tarsal segments bearing 3 hairs except the single species, viz, *dicentrae* BASU which possesses first tarsal segment with 4 hairs of which 2 of these hairs are

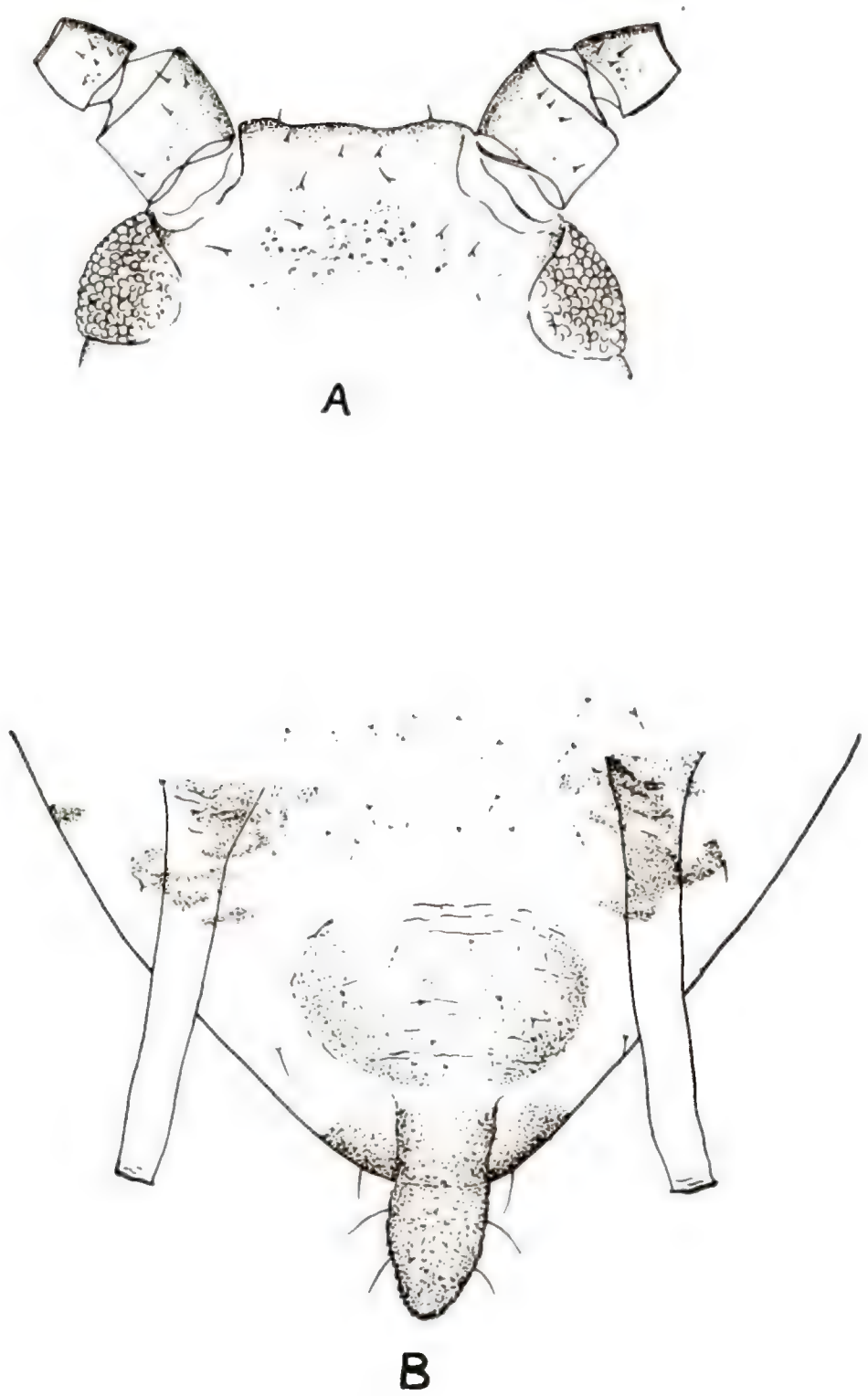

Fig. 2. *Scleromyzus corylopsis* (BASU, GHOSH & RAYACHAUDHURI), Apterous viviparous female. A. Head, B. Posterior portion of abdomen.

short, stout and blunt. So the new subgenus *Paraneomyzus* is erected under *Neomyzus* with *dicentrae* BASU. It may be mentioned here that *Neomyzus* treated as a subgenus of *Aulacorthum* by other workers has been given the generic status.

Type species : *Aulacorthum* (*Neomyzus*) *dicentrae* BASU (1967).

Scleromyzus gen. nov.

The characters for the monotypic genus is not provided here as the type species, *Myzus corylopsis* has recently been described in detail by BASU *et al.* (1973). So the species characters are also the generic characters. However, the justification for the erection of the new genus is discussed here.

The species with which the new genus is erected was sent to DR. D. HILLE RIS LAMBERS, Holland for comments when he wrote "species and genera not known to me, rough head, presence of pleural intersegmental sclerites exclude *Acyrthosiphon* but suggest relationship to *Myzus*. I do not suggest genus or species and shall file this with the 14 slides without genus name. An alate might help to solve the classification problem" DR. HILLE RIS LAMBERS was quite correct in suggesting the relationship with *Myzus* but the very ill developed lateral frontal tubercles, sparsely spinulose head (Fig. 2A) and the post siphuncular sclerite (Fig. 2B) found in the species distinguish the new genus from *Myzus*.

The new species *corylopsis* could possibly be considered under *Micromyzus* but then again one should expect to find well developed lateral frontal tubercles, strongly spinulose head and absence of post siphuncular sclerite.

The other genera, viz., *Eomyzus* TAKAHASHI and *Eumyzus* TAKAHASHI might be looked upon as close to the new genus since in both these genera the apterae viviparae have spinulose head, lack secondary rhinaria and may or may not have segmental sclerites on abdomen. The present genus can, however, be distinguished from *Eumyzus* by the low lateral frontal tubercles and absence of tubercles at bases of the dorsal abdominal hairs which are short and from *Eumyzus* by the presence of sclerites on abdomen, smooth siphunculi and very short abdominal hairs.

Type species: *Myzus corylopsis* BASU, GHOSH and RAYCHAUDHURI, 1973

Distribution: India.

Xenomyzus scabripes spec. nov.

Apterous viviparous female: Body about 1.30—1.55 mm long with 0.67—1.09 mm as the maximum width. Head densely spinulose both dorsally and ventrally; lateral frontal tubercles low but distinct with scabrous rounded apices; frons rather concave; dorsal cephalic hairs short with blunt apices ventral one rather long with acuminate apices. Antennae 6-segmented, shorter than to nearly as long as body; segments I & II spinulosely scabrous; flagellum densely imbricated with few spinules on basal 0.50 portion of segment III; flagellar hairs short, sparse with blunt apices, the longest one on segment III about 0.17—0.37 × the basal diameter of the segment; secondary rhinaria absent; p.t. about 3.40—3.80 × the base of segment VI; primary rhinaria non ciliated. Rostrum extends upto mid coxae; u.r.s. normal, about 0.93—1.05 × h.t.2 and bears a pair of secondary hairs; prothorax free; segmentation between meso-, and metathorax and on first six abdominal segments obsolate. Thoracic segments dorsally wrinkled and ventrally spinulose particularly on prothorax. Mid thoracic furca sessile. Abdominal dorsum wrinkled upto segment 6, rest with transverse spinular striae muscle plates sometimes present pleurally on antesiphuncular segments, dorsal abdominal hairs sparse, short with blunt apices, the longest

one on anterior tergites about 0.15—0.25× the basal diameter of antennal segment III; segments 7 and 8, each with a pair of long hairs having acuminate apices which are about 0.40—0.55×the mentioned diameter. Abdominal spiracles on sclerotic areas, those on segments 6 and 7 much closer than those on segments 5 and 6. Siphunculi brown, subcylindrical with distinctly broad base which on outer margin slightly pushed inwards, remarkably narrowing apical (basal diameter being about 5.0×the diameter at apex), with dense spinular imbrications all over, with short, sparse and blunt hairs, without apical flange and with the pore placed slightly obliquely, about 0.20—0.25× the body. Cauda pale, blunt, sometimes slightly constricted near the base, about 0.30—0.42×the siphunculi and bears 4–5 hairs. Femora dorsally densely imbricated and ventrally spinulosely scabrous; tibiae with spinular imbrications; tarsi with normal imbrications; hairs on legs short with blunt to acuminate apices, F.T.C. 3,3,2.

Measurements of the holotype in mm: Length of body 1.44, width 0.75; antenna 1.11, segments III:IV:V:VI 0.22:0.14:0.13: (0.10+0.34); u.r.s. 0.08; h.t.2 0.08; siphunculus 0.36; cauda 0.12.

Alate viviparous female: Head brown, lateral frontal tubercles hardly indicated. Antennae concolorous with the head, about 0.80—1.05×the body; segment III with 29–32, segment IV with 9–13 and segment V with 6–10 irregularly distributed protuberant secondary rhinaria; longest hair on segment III about 0.45—0.75×the basal diameter of the segment; p.t. about 3.30—5.15×the base of segment VI. Abdominal dorsum pale, with marginal spinular pigmented patches on each of segments 2–5 and a diffuse brown broad transverse pigmented bar on each of 7th and 8th tergites; legs brown, femora dorsally imbricated and ventrally spinulose; tibiae smooth. Wing venation normal. Otherwise as in apterae viviparae.

Measurements of one specimen in mm: Length of body 1.65, width 0.77; antenna 1.74, segments III:IV:V:VI 0.37:0.24:0.24: (0.11+0.54); u.r.s. 0.08; h.t.2 0.08; siphunculus 0.26; cauda 0.10.

Holotype: Apterous viviparous ♀, India: West Bengal: Darjeeling: Kurseong, 10.i. 1971, from *Polygonum moli*, coll. M. R. GHOSH, Paratypes many apterous viviparous ♀♀, alate viviparous ♀♀ and nymphs, for same collection data same as for the holotype and some others collected from different localities of Arunachal, Meghalaya, Sikkim and West Bengal on different dates and from different species of *Polygonum*.

Remark: The new species comes close to *polygoni* but differs in having longer dorsal cephalic hairs, shorter body, rather short processus terminalis, dark siphunculi besides more scabrous femora and tibiae.

Re-examination of all the material previously reported as *Metaphorodon polygoni* reveals that only 6 apterae collected on *Polygonum barbatum* and *Polygonum* sp. in West Bengal are true *polygoni* and rest belong to this new species, *Scabripes*. *Metaphorodon* has been considered here as a synonym of *Xenomyzus*. This idea is also expressed by HILLE RIS LAMBERS (1969) and MIYAZAKI (1971).

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Entomon, 1976, *Vol.* 1, *No.* 1, *pp.* 67 *to* 70.

MORPHOLOGICAL VARIATIONS IN THE LOCAL POPULATIONS OF THE SOIL COLLEMBOLA *PROISOTOMA (CLAVISOTOMA) FITCHIOIDES* (DENIS 1947) (INSECTA) IN KERALA

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Morphological variations in *Proisotoma (Clavisotoma) fitchioides* collected from soils of two neighbouring localities are presented. Form A obtained from one locality was well pigmented while form B from the other locality was poorly pigmented in comparison with the former. Variations exist in the mean body length and in the mean ratios, antennal length/head diagonal, trunk/tibiotarsus III, trunk/furcula and manubrium dens + mucro. Application of 't' test showed that while the difference in the mean body length between form A and form B was insignificant, the difference in the mean of the ratios was highly significant. Together with the reduction in the pigmentation of the body a tendency for the reduction in the length of the appendages in relation to the body length and a reduction in the number of a setae in the trunk segments was noted in form B. The patchy distribution of the species was thought to be responsible for maintaining the kind of variations observed in the species studied.

INTRODUCTION

Proisotoma fitchiodes was described by DENIS (1947) from Nilgiri hills in India. YOSII (1966) recorded the species from Burma and from Bombay, transferred it to *Subisotoma* and gave the genotypical characters of the genus. According to ELLIS (1970) the genus *Subisotoma sensu* YOSII is not acceptable as it excludes *S. pusilla* (SCHAEFFER) from the definition, *pusilla* being the type species of the genus. Consequently ELLIS (1970) synonymized *Subisotoma* with *Clavisotoma* PALISSA (1964) a subgenus of *Proisotoma,* redefined *Clavisotoma* and designated *Proisotoma tuberculata* STACH (1947) as the type species of *Clavisotoma.* He also listed 25 species belonging to this subgenus. Following ELLIS (1970) *Subisotoma fitchiodes* (DENIS) is considered here as *Proisotoma* (*Clavisotoma*) *fitchiodes.*

Proisotoma (*Clavisotoma*) *fitchiodes* (DENIS) seems to have a limited distribution in Kerala. Earlier studies of Collembola from Kerala (PRABHOO, 1971 a, b) did not reveal the presence of this species in the localities sampled. It was subsequently recorded from two localities sampled near Trivandrum viz., Kariavattom and Kesavadasapuram, which are about 8 km apart. Study of the individuals of this species from the two localities mentioned above revealed the existence of some degree of differentiation of the two populations which is the subject of the present contribution.

MATERIAL AND METHODS

Several individuals from a grass plot at Kariavattom were obtained from the surface of water collected in a pit after a heavy rain fall in June 1972. From humus soil around a coconut plant at Kesavadasapuram 13 examples were collected in June 1972. The individuals were fixed in 80% alcohol and were examined in GISIN's (GISIN, 1960) temporary mountant. Measurements were made of ten individuals each from the two populations using an eye-piece micrometer. The 't' test was used to measure the significance of the difference in the mean values as the sample was small. For statistical test the procedure given in MORONEY (1956) was followed.

RESULTS

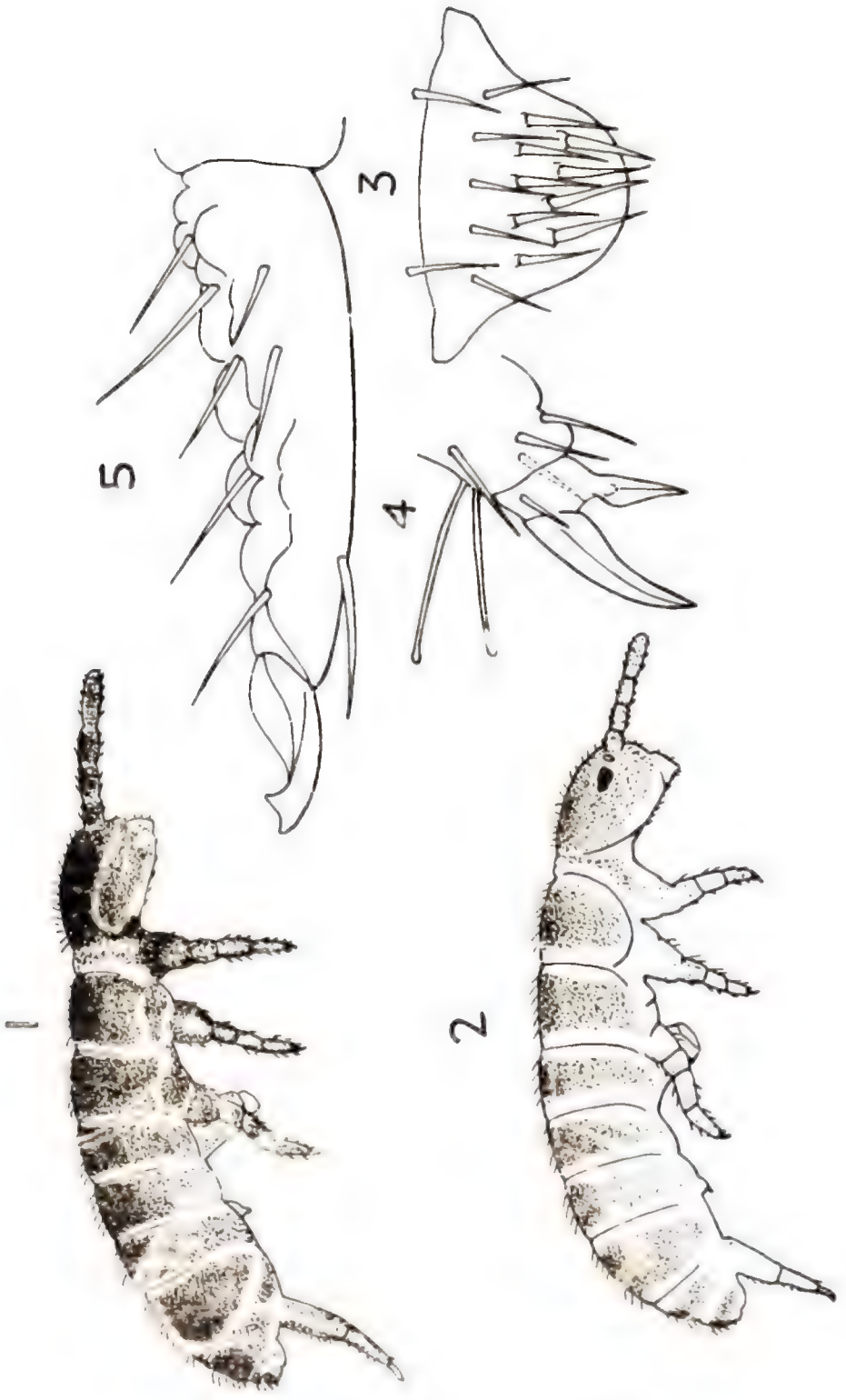
For convenience the individuals in the population of Kariavattom and Kesavadasapuram were designated forms A and B respectively. The colour pattern was strikingly different. Form A showed dirty white ground colour with bluish black pigment uniformly distributed on the dorsal and lateral sides of the body (Fig. 1). Intersegmental areas were almost devoid of pigment. On the head ocellar fields were not clearly distinguishable from neighbouring pigmented regions. Some individuals which could be definitely identified as females, because of the presence of eggs in the abdomen, were lighter in colour and somewhat intermediate in the colour scheme between that described above and that of B. In form B (Fig. 2) the pigment had low intensity but it was distributed in a manner more or less similar to that of A, there being much less pigment laterally on the trunk segments and on the legs and furcula. The ocellar fields were clearly distinguishable from the neighbouring pigment on the head. Ant. I with a complete row of short setae and one sense rod ventrally. The second row with only a short seta in A but in B there were at least two setae. Ant. II with two complete rows of setae and a proximal incomplete row with two setae. Ventrelly there was also a sense rod. Ant. III organ with 2 short sense rods and 2 sense setae. Ant. IV apically without sensory papilla but with 12–15 subapical sense rods, some of which were well differentiated. Labrum (Fig. 3) with 2/5, 5, 4 setae of which the distal two rows were papillate. Eyes 8+8. Pao elliptical and slightly longer than the diameter of the anterior ocellus. Claw (Fig. 4) without teeth. Unguiculus with fairly developed lamellae. Tibiotarsal tenent hairs 1, 2, 2, which were dinstictly knobbed at the tip. Trunk covered with short setae. Abd. I–III with 6, 5,5, or 5, 5, 5, rows of setae in A. while in B there were only 5, 4, 4 or 4, 4, 4 or 4, 4, 3 rows of setae. Ventral tube without anterior setae, with 1+1 posterior setae and the lateral flap with 3 or 4 setae in A but only 3 setae in B. Cami tenaculi with 3+3 dents and 1 seta on the corpus. Manubrium dorsally with about 20 pairs of setae and ventrally without seta. Dens (Fig. 5) dorsally with about 10 integumentary swellings and 7 setae arranged as 1, 2, 2, 1, 1 and a prominent subapical ventral seta. Mucro bidentate, clearly demarcated from the dens and with the inner lamella broadly rounded near the base.

The range of measurements of the body parts, the mean, the standard deviation (S), the 't' value (t) and the probability (P) are given below.

The body length in form A ranged from 1.065 – 1.119 mm (mean = 1.084, S =19.19) and in form B the range was 1.031 – 1.116 mm (mean = 1.064, S = 29.9); t = 1.71 and P between 20% and 10%. The ratio Antennal length/Head diagonal in A was 1 – 1.297, mean = 1.0618, S = 0.0954; in B 0.6884 – 0.9104, mean = 0.8076, S = 0.0848; t = 6.22, P < 0.1%. The ratio Trunk/Tibiotarsus III in A 11.15 – 11.80, mean = 11.483, S = 0.1714; in B 14.50 – 16.66, mean = 15.347, S = 0.6754; t = 16.65, P< 0.1%. The ratio Trunk/Furcula in A 3.819 – 4.097, mean = 3.120, S = 0.4262; t = 8.73, P<0.1%. The ratio Manubrium/Dens + Mucro in A 0.9080 – 1.0180, mean = 0.9533, S = 0.03; in B 1.104 – 1.153, mean = 1.123, S = 0.17; t = 14.76, P<0.1%.

DISCUSSION

The information presented above indicates that individuals from two localities which are separated only by a short distance from each other showed morphological differences some of which are well marked. Variability of colouration was one of intensity and with



Figs. 1–5. *Proisotoma* (*Clavisotoma*) *fitchioides*
Fig. 1, form A in profile; Fig. 2, form B in profile; Fig. 3, labrum of form A; Fig. 4, hind claw of form A; Fig. 5, dens and mucro of form A (pigment not represented)

a tendency to produce intermediate forms. The variability in length was not significant. On the other hand difference in mean of the ratios of body parts was found to be highly significant. Together with a reduction in the body pigment in form B it was found that the appendages had shown a tendency to become shorter in relation to the total length of the body. Further there was also a reduction in the number of the setae in the thoracic and abdominal segments. In spite of the differences mentioned above there was little doubt that the two populations belonged to the same species. The key characters of the species are the number and distribution of setae on the dens, presence of clavate tenent hairs on tibiotarsi of legs and the claws without teeth, which were found to be similar to the condition described by DENIS (1747). As stated above the species has a limited distribution in Kerala. Even at Kariavattom it was confined to certain fields and occasional samples of soil collected from a locality between Kariavattom and Kesavadasapuram did not reveal the species. The variations were therefore of the kind that could be expected when panmictic populations of a species were distributed in territories which were not continuously inhabited. These are to be distinguished from variations like those found, for instance, by PRABHOO (1971 b) in the colour pattern of *Lipothrix indicus* and by GROW & CHRISTIANSEN (1974) in the chaetotaxy of *Friesea grandis* which were likely to be due to polymorphism. It may also be mentioned here that *Proisotoma* (*Clavisotoma*) *canituda* (SALMON) (*Subisotoma canituda* (SALMON) *sensu* PRABHOO (1971 a) which was found to be widely distributed in Kerala, showed little morphological variations (PRABHOO, 1971 a). The shortening of appendages and reduction in the body setae shown by form B were the type of modifications of the body suitable for penetrating into deeper layers of the soil.

Proisotoma (*Clavisotoma*) *fitchioides* also showed variations which were of a geographic nature. Thus the mean length of the body recorded by DENIS (1947) was 0.825mm for material from Nilgiris. Individuals from Bombay and Burma had a length of about 1.4 mm (YOSII 1966) while the present examples were intermediate in size. Further, in Yosii's material there were two clavate tenent hairs on leg I and the three hind ocelli showed reduction while in the examples from Nilgiris and from Kerala there was only one clavate tenent hair on leg I and the ocelli appeared to be normal in the examples studied.

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ASPECTS OF HOST PREFERENCE AND SUCCESSION IN THRIPS INFESTING *RUELLIA TUBEROSA*

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Polyphagous and restricted oligophagous species when they inhabit a host plant with a restricted flowering season, tend to show ecological succession and interspecific competition. The periodicity of occurrence and abundance of six species of thrips inhabiting the flowers of *Ruellia* as evaluated from their population dynamics for a period of two years, indicated that, while the polyphagous species were always primary or secondary colonizers, the restricted oligophagous species were always tertiary inhabitants. The frequency distribution or the number of thrips per flower per day for two years is discussed along with the nature of the host range of the primary and secondary species.

INTRODUCTION

Information relating to fluctuations in natural populations of anthophilous Thysnoptera is given by DAVIDSON & ANDREWARTHA (1948 a, b), ANDREWARTHA & BIRCH (1954) and SMITH (1953) on *Thrips imaginis* BAGNALL infesting roses in Australia, RAIZADA (1963) on *Microcephalothrips abdominalis* CRAWFORD on Marigold in India, WARD (1966) on *Thrips validus* UZEL on varied hosts in England and LAUGHLIN (1970) on *Isoneurothrips australis* BAGNALL in Australia. KUDO (1971) provides interesting data on the population fluctuations of a number of species inhabiting nine different species of flowers, laying particular stress on the phenology, frequency distribution and diurnal variations of different species. Results presented herein relate to a detailed analysis of not only the population fluctuations of different species of thrips infesting the solitary flowers of *Ruellia tuberosa* but also to aspects of ecological succession and interspecific competition.

Six species of thrips inhabiting the flowers are *Haplothrips gowdeyi* (FRANKLIN), *Frankliniella schultzei* PRIESNER, *Megalurothrips distalis* KARNY, *Thrips hawaiiensis* MORGAN, *Tusothrips aureus* (MOULTON) and *Micothrips fasciatus* ANANTHAKRISHNAN. Of these the first four species are typically polyphagous being recorded on a variety of hosts, while the last two have been reported only from *Ruellia tuberosa* in India. The periodicity of occurrence and abundance of all the six species present considerable variations.

MATERIALS AND METHODS

In an attempt to study the fluctuations of different species at Madras data was collected for the years 1972 and 1973 from June to October, with collections being made every fourth day from among 30 flowers obtained at each collection. Care was taken to choose flowers of same age and from same area.

RESULTS AND DISCUSSION

The thrips populations within *R. tuberosa* showed a remarkable succession resulting in the displacement of one species by another, a feature primarily dependent upon not only the seasonal periodicity, but also

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the host range and colonizing ability of the respective species of thrips. On the basis of their relative duration within the flowers the thrips species may be designated as *primary*, *secondry* and *tertiary*. *H. gowdeyi* and *F. schultzei* being the first to appear within the flowers and occurring as they do throughout the flowering season, are the primary species *M. distalis* and *T. hawaiiensis* representing the secondary species occur comparatively in fewer numbers than the primary species. These secondary species also occur throughout the period of study but display considerable irregularity in their population trends. Equally characteristic of the secondary species is their belated appearance within the flowers after a severe environmental stress. The tertiary species represented by *T. aureus* and *M. fasciatus* occurring in fewer numbers than the primary or secondary are present only for a short period and appear highly host specific. All the species never occur together in a single collection in view of their graded periodicities and of the tertiary species only one is found to occur at a time.

The rise and fall in numbers of different species appear independent of each other often following the conditions of the environment. In both 1972 and 1973 the highest temperature recorded for the period of study was noted in the month of June (27.4°C – 37.0°C) in 1972 and July (27.9 – 36.7°C) in 1973. The two primary species had their peaks of abundance in June-July and their minimum numbers were noted in September-October when the mean temperature was only 24.4°C – 30.6°C and 24.8°C–32.0°C respectively. Relative humidity in June–July was lowest being 67% in 1972 and 61% in 1973 and highest in September-October viz. 86% in 1972 and 84% in 1973. Among the secondary species *T. hawaiiensis* had a numerical abundance over *M. distalis* in 1972. These two species had their own respective periods of abundance or sparseness irrespective of those of primary species. However, *M. distalis* overnumbered the primary species in 1973. The increase in numbers was so high that it practically replaced *H. gowdeyi*, the primary species during one phase of collection. In spite of this attribute they are treated only as secondary species because of their restricted ability for colonization after an environmental stress. An aspect of interest relates to *M. fasciatus* which occur during early weeks of collection while *T. aureus* appeared at the last phase of collection. To be more specific *M. fasciatus* reached its peak during May-June indicating a preference to low humidity and high temperature, while *T. aureus* reached its maximum numbers in August-September during conditions of higher humidity and lower temperature. The number of secondary species had also a regulating effect on the abundance or sparseness of primary species. This was particularly true with regard to one secondary species viz. *M. distalis*.

Sex Ratio

In 1972 and 1973 there was an overabundance of females in thrips population (Table 1 a, b). The individual species also showed the same trend, although in 1973 the sex ratio of secondary species was slightly different from that of 1972. *H. gowdeyi* had the maximum male/female ratio (0.29 and 0.28) in both years. Among the secondary species *M. distalis* recorded the highest male/female ratio (0.26 and 0.28) for 1972 and 1973 respectively. The males of secondary species were rare in the collections of 1972 and 1973 but *T. aureus* showed a male/female ratio of 0.71 which is negligible compared to that of other species, thereby showing a preponderance of females in the population of tertiary species.

Observations on the frequency distribution (number of thrips per flower per day) showed

TABLE 1a. Relative abundance of adult thrips in *Ruellia tuberosa* for the year 1972
Number of individuals collected

Species	Females	Males	Females plus Males	% to total number	Ratio of male/ female
Haplothrips gowdeyi ..	249	73	322	39.22	0.29
Frankliniella schultzei ..	235	8	243	29.59	0.340
Megalurothrips distalis ..	34	9	43	5.22	0.26
Thrips hawaiiensis ..	85	1	86	10.47	.011
Tusothrips pseudosetiprivus ..	102	0	102	12.42	0/102
Micothrips fasciatus ..	25	0	25	3.04	0/25
Total ..	730	91	821		

TABLE 1b. Relative abundance of adult thrips in *Ruellia tuberosa* for the year 1973
Number of individuals collected

Species	Females	Males	Females plus Males	% to total number	Ratio of Males/ Females
Haplothrips gowdeyi ..	210	59	269	24.003	0.2809
Frankliniella schultzei ..	379	16	295	38.46	0.42
Megalurothrips distalis ..	375	78	453	41.98	0.208
Thrips hawaiiensis ..	42	2	44	4.07	0.47
Tusothrips pseudosetiprivus ..	14	1	15	1.39	.071
Micothrips fasciatus ..	3	0	3	.278	0/3
Total .	923	156	1079		

that it presented variations for each of the species during different months and for the same species in the same month (Figs. 1 & 2). *H. gowdeyi* presented the highest frequency distribution in 1972 being more conspicuous in June. *F. schultzei* was the next populous species with others following at different levels. In 1973 the frequency of the primary species varied greatly due to the sudden spurt in the population of *M. distalis*. The number of *M. distalis* per flower per day was highest during the period 13.8.1973 to 22.8.1973. This abudance was not only due to the absence of other species but also to

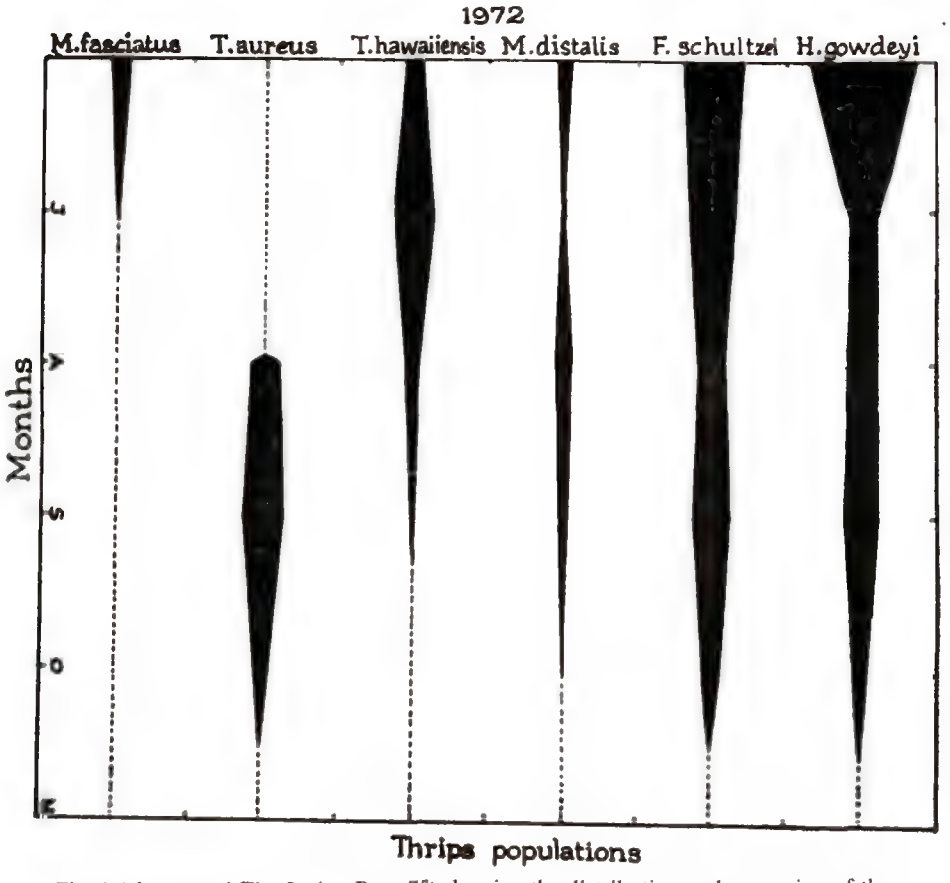
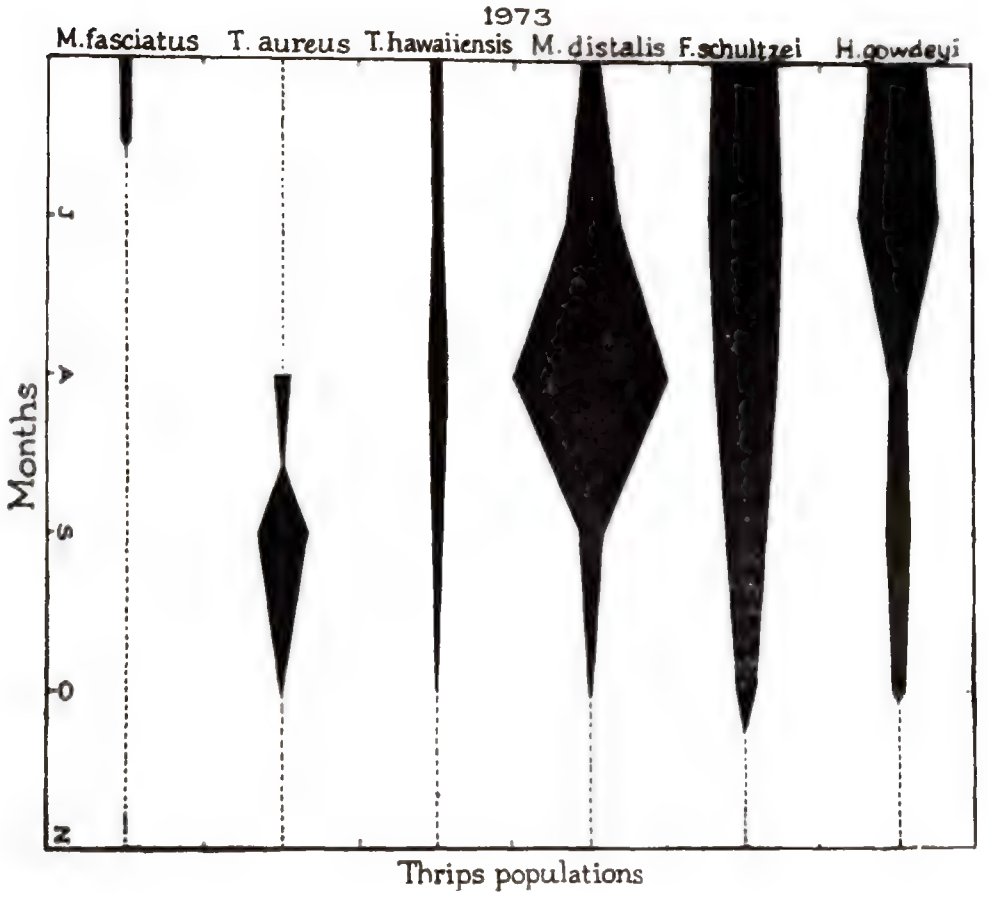


Fig. 1 (above) and Fig. 2 (on Page 75) showing the distribution and succession of the species during different months.

the exclusion of the primary species, an ideal example where other species are overshadowed by mere numerical abundance — an instance of interspecific competition. Irrespective of whether they are primary species or tertiary species, all of them appear to be at the mercy of density independent factors especially temperature and humidity. This is further confirmed by the fact that at no time of collection throughout the two year period was there a predator or prey observed and the flowers have only a short duration about 2–3 days. That the control is primarily density independent is illustrated by the differential periods of abundance or sparseness. For example, in June-July there was an abundance of the primary species and a sparseness of secondary species. The intermediate conditions afforded by August–September saw a richness of primary species. But in 1973 *H. gowdeyi* was relatively sparse. Among the secondary species *T. hawaiiensis* was conspicuously sparce in 1972 and 1973 but *M. distalis* which was found only in fewer numbers in 1972 was very abundant in 1973. With regard to the tertiary species *T. aureus* had its sparseness, richness and decline in August–September and *M. fasciatus* was totally absent in these two months. But the month of June had seen a reverse situation



with *M. fasciatus* being abundant and *T. aureus* totally absent. That the drier conditions of the atmosphere encourage abundance of thrips on the flowring plants are discussed by LAMB (1965) and LEWIS (1973). VON OETTINGEN (1942) similarly points out that wet conditions had a scarcity of thrips. Comparison of data of 1972 and 1973 (Fig. 3) would indicate that this abundance is especially conspicuous with regard to primary and secondary species.

The collections of 2 years indicated that the larvae of primary and secondary species were frequent in occurrence and those of tertiary species were relatively sparce. *H. gowdeyi* larvae appeared in maximum numbers among the primary species. During the phase of collection, when *M. distalis* temporarily excluded the primary species from the environment, it was interesting to observe that not even one larva of *M. distalis* was observed.

H. gowdeyi, an exclusively anthophilous thrips was present throughout the period of study. Similarly, *F. schultzei* was observed in all eollections although in fewer numbers. Their abundance even in situations when climatic factors were extreme, mark them out as the most successful inhabitants and euroecious species. The failure of *M. distalis* and *T. hawaiiensis* to do so and their tendency to increase in mumbers only when

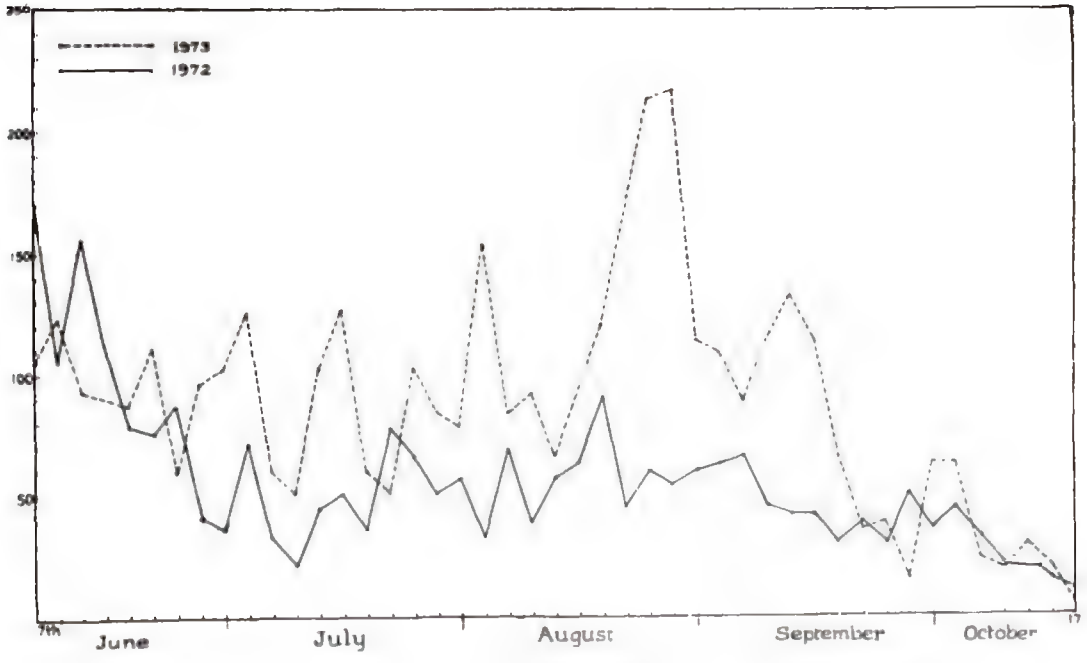


Fig. 3. Total population counts in fortyfive flowers made every third day from 7th June to 17th October.

proper environmental conditions arise mark them out as the secondary species.

Taking into consideration that no notable predator was observed the only density dependent factor evident is the interspecific competition between different species. As MILNE (1957) points out it is a very important density dependent factor since the rise and fall of populations is controlled by it. The principal environmental factors controlling thysanopteran populations seem to be density independent ones–especially temperature and humidity, Since rainfall is sparse during these months it is never an important factor. DAVIDSON & ANDREWARTHA (1948 a, b) and ANDREWARTHA & BIRCH (1954) considered from their srudies on *Thrips imaginis* on roses that rainfall and temperature can together control thrips populations when density dependent factors are totally absent. But a situation devoid of density dependent factors is remote and hence one has to agree with MILNE (1957) who states that fluctuations are the result of density independent factors and oscillations due to density dependent ones, and it is the interaction of the two factors that regulates a population.

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PERSISTENCE OF PHORATE AND ITS RESIDUAL TOXICITY TO *APHIS CRACCIVORA* KOCH. IN COWPEA

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Enzymatic assay of internal residues of phorate in cowpea *Vigna sinensis* following application of the insecticide as granules with seed at 1, 2 and 3 kg a.i. / ha showed that residues of the toxicant persisted within the plants upto 49 days following the application. On the 56th day there was no residue of the insecticide either in the leaves or in the pods. The mortality of *Aphis craccivora* exposed to the plants treated with the granules was 100 per cent upto 14 days with 1 and 2 kg a.i. / ha and upto 21 days with 3 kg a.i. / ha. The mortality showed a gradual reduction corresponding to the reduction of the internal residue of the insecticide, there being no mortality on 56th day of insecticide application. Application of phorate granules at 2 kg a.i. / ha, with the seeds is considered an effective and safe method for protecting cowpea from infestation by *A. craccivora* upto 8 weeks of its growth.

INTRODUCTION

Phorate is widely recommended for the control of sucking pests affecting pulse crops (NEEL *et al.*, 1967; GOULD *et al.*, 1969; HAGEL, 1970; CHANG, 1971; NARESH & THAKUR, 1972; MATHUR *et al.*, 1974; SHASHI VERMA, 1975; BHATTACHARJEE *et al.*, 1975). In recommending phorate for insect control on edible crops it is necessary to ascertain the persistence of the toxicant within the plants. This has already been done with various pulse crops in various centres (SHASHI VERMA, 1975; SHASHI VERMA & PANT, 1975; BHATTACHARJEE *et al.*, 1975).

The present paper embodies the results of estimations of phorate residues made in *Vigna sinensis* grown in the red soil of Kerala State. The variety used is a high yielding cultivar called 'Philippines' which is generally cultivated for vegetable purpose. Studies on the residual toxicity of the insecticide to *Aphis craccivora*, the most important pest of the crop, have also been made.

MATERIALS AND METHODS

The cowpea plants were raised in pots (30 cm x 30 cm) using 20 kg of soil per pot. The soil was collected from a spot known to be uncontaminated by insecticide applications. The soil in each pot was mixed with 200 g of farm yard manure. A single seed was sown in each pot. The different doses of phorate in the form of granules were applied together with the seeds in quantities calculated for each pot. Pots were watered every morning and evening. Twentyfive days after sowing the plants were fertilised with 2g ammonium sulphate per pot.

The internal residues of phorate within the plants were determined chemically following the enzymatic methods of SUTHERLAND *et al.* (1964). For these assays 10 g samples of apical portion of the plants under the various treatments were collected at weekly intervals and extracted for the residues. Residues of the toxicant in the pods also were assessed.

To study the toxicity of the internal residues of phorate to *Aphis craccivora* adults of the aphid were exposed to apical parts of the plants under the different treatments on the same occasions as the plant parts were collected for chemical assays of the residues and mortalities observed. The apical parts of the plants were cut out placed in specimen tubes

TABLE 1. Residue of phorate in cowpea in ppm at different intervals

Doses of phorate (kg. ai./ha)	Intervals in days							
	7	14	21	28	35	42	49	56
1 ..	24.40	27.00	16.4	8.2	0.36	0.30	0.17	ND
2 ..	25.04	33.00	24.4	16.2	0.56	0.36	0.23	ND
3 ..	26.20	43.00	32.0	21.9	0.65	0.54	0.30	ND

ND — Not detected.

TABLE 2. Percentage mortality of *A. craccivora* on cowpea at different intervals after phorate application

Doses of phorate (kg. ai./ha)	Intervals in days							
	7	14	21	28	35	42	49	56
1 ..	100	100	80	60	46	17	0	0
2 ..	100	100	83	83	50	37	0.3	0
3 ..	100	100	100	96	60	37	1.0	0

TABLE 3. ANOVA (after angular transformation)

Source	S. S.	D. F.	M.S.S.	F
Total ..	62162.27	62		
Block ..	268.94	2	134.47	2.46
Treatment ..	1586.57	2	793.29	14.52**
Period ..	56293.27	6	9382.21	171.77**
Treatment x period ..	1828.56	12	152.38	2.79**
E ..	2184.93	40	54.62	

** Significant at 1% level

CD for comparing treatments = 4.609

.. .. periods = 7.041

.. .. combinations = 12.195

Means : $T_1 = 51.53$, $T_2 = 59.97$ $T_3 = 63.80$

Periods : $p_1 = 90$ $p_2 = 90$ $p_3 = 73.20$ $p_4 = 67.85$

$p_5 = 46.31$ $p_6 = 32.12$ $p_7 = 2.55$

and adults of the aphid reared on plants in field cages released on them. Mortality counts were made 24 hours after exposure on the plants.

RESULTS

Table 1 gives the amounts of phorate persisting within the plants at different intervals after application and when applied at different doses. It may be observed that with each dose there is an increase in the insecticide contents of the plants upto 14th day after the application. From the 14th day a steady decrease in the internal residues of the toxicant is in evidence. However, a very abrupt and drastic reduction in the insecticide residues is registered between the 28th and the 35th day of insecticide application. There is no detectable residues on the 56th day in any of the doses under study. Estimations of internal residues of pods on 56th day also did not show any detectable residues of the insecticide.

Table 2 gives toxicity of internal residues of phorate in the cowpea plants to *Aphis craccivora* exposed to the plants at different intervals after the insecticide application. It may be observed that there is cent per cent

TABLE 4. Per cent mortality of *Aphis craccivora* corresponding to internal residues of phorate in cowpea

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Phorate residue (ppm)	43.0	33.0	32.0	27.0	26.2	25.04	24.4	24.4	21.9	16.4	16.2	8.2	0.65	0.56	0.54	0.36	0.36	0.30	0.30	0.23	0.17
Per cent mortality	100	100	100	100	100	100	100	83	96	80	83	60	60	50	37	46	37	17	1	0.3	0.0

mortality of the aphids upto 2 weeks in the case of doses of 1 and 2 kg a.i./ha and upto 3 weeks in 3 kg a.i./ha. Thereafter gradual reduction in mortality is in evidence. There is, however, an abrupt and substantial reduction in the mortality on the 35th day after the insecticide application; mortality becomes insignificant on the 49th day.

The analysis of variance table (Table 3) shows that there is no significant difference in the toxicity of phorate residues to the aphid when used at the dosages of 2 and 3 kg a.i./ha. There is, however, significant difference between the mortality caused by the dose of 1 kg a.i./ha and that of the two higher doses. As regards the mortality variations with reference to intervals after insecticide application the analysis of variance shows that in general the mortalities differ significantly between the different occasions.

DISCUSSION

Results of studies presented show that residues of phorate applied in soil with the seeds persist within cowpea plants upto a period of 49 days following application. On the 56th day of treatment no residue is detected even at the highest dose of 3 kg a i. per hectare. Analysis of pods also has indicated absence of any residue on the 56th day. The pods will be ready for harvesting usually at the 8th week following sowing. As the acceptance for phorate on food crops is on "no residue at harvest basis" application of the granule together with the seeds as has been done in the present studies is a safe treatment for cowpea. Comparable results have been reported by previous workers on other pulse crops. Thus SHASHI VERMA (1975) and SHASHI VERMA & PANT (1975) could detect residues of phorate in 'moong' and 'arhar' upto a period of about 50 days and 62 days respectively. BHATTACHARJEE *et al.* (1975) using phorate granules before seed placement

and as side dressing 4 weeks after initial application @ 2.5 gm/meter row, observed no residues in leaves, straw or grain of soybean at the time of harvest. The lower phorate contents of plants on the 7th day than on the 14th day appears to be due to lack of proper root system on the 7th day enabling absorption of the toxicant.

Table 4 gives the mortalities of *A. craccivora* corresponding to the different internal residues of phorate. It is seen that the mortality of the aphid is cent per cent for an internal residue of 24.4 ppm and above, of the toxicant. As the internal residue contents decrease below 24.4 ppm the mortality of the aphid also decreases correspondingly. On the 56th day of insecticide application there is no detectable residue in the plant and there is no mortality in the aphids also.

Application of phorate granules with seeds is seen as an effective and safe method for protecting cow pea plants upon a period of 7 weeks. A dose of 2 kg a.i./ha has proved itself effective for the purpose. These findings have special significance as the cow-pea variety under studies is one which is used as a vegetable and is harvested continuously.

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A MODIFIED TECHNIQUE FOR GRASSHOPPER CHROMOSOME PREPARATIONS

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Making use of colchicine-hypotonic-flame dry technique, hepatic caeca of acridids and tettigoniids have been used as the source of material for the preparation of somatic chromosomes.

INTRODUCTION

Mostly testes material is used to study chromosome number, structure and behaviour. Occasionally ovariole walls (JOHN & HEWITT, 1966) and neuroblasts (HEWITT & JOHN, 1971) have been used to study the somatic chromosome architecture. The authors of this paper have developed a technique using hepatic caeca of the acridid–*Acrida turrita* and a tettigoniid-*Euconcocephalus incertus*.

MATERIALS AND METHODS

The animals collected from Manasagangotri, Mysore, were injected 0.1 ml of 0.05% colchicine and sacrificed after 4 hours. The hepatic caeca were dissected out and treated with hypotonic 0.9% sodium citrate solution at room temperature for 1 hour. The material was fixed for 3 hours in 1:3 acetic/methanol with three changes of the fixative. The material was minced well on a clean slide and centrifuged at 750 RPM for 5 minutes. The supernatant was discarded and the cell button resuspended with fresh fixative. Cell suspension was dropped on alcohol-cleaned slides and were flame dried. The slides were stained with Giemsa, dried and mounted in DPX.

RESULTS AND DISCUSSION

The photographs (Figs. 1 and 2) show the clarity of the chromosome structure including the position of the centromere. The preparations obtained from the hepatic caeca are far better than those prepared from the testes (Figs. 3 and 4). Based on the cytology of the testicular cells of *Acrida turrita* it was believed that the chromosomes were acrocentric, but the somatic chromosome preparations from the hepatic caeca clearly show that they are telocentric. Further, the clarity of the chromosome size and position of the centromere are better expressed in the hepatic caeca preparations than in intestinal epithelium or Malpighian tubules. In addition, it was observed that the output of metaphase plates in the material under discussion outweighs those from other tissues. This is an added advantage. Further when one is studying metaphase chromosomes from neuroblasts, one has to get developing embryos which is ordinarily difficult. This high number is useful for comparative and mitotic index studies. Moreover, the usage of hepatic caeca from female grasshoppers overcomes the difficulties involved in getting chromosome preparations from ovarioles of orthopterans which are full of yolk. The authors opine that this technique would be easy and useful for all the orthopteran chromosomologists.

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Fig. 1. Mitotic metaphase of *Acrida turrita* (2n $\overset{+}{O}$=24, hepatic caeca).

Fig. 2. Mitotic metaphase of *Euconcocephalus incertus* (2n $\overset{+}{O}$=22, hepatic caeca)

Fig. 3. Spermatogonial metaphase of *Acrida turrita* (2n $\overset{\uparrow}{0}$=23).

Fig. 4. Spermatogonial metaphase of *Euconcocephalus incertus* (2n $\overset{\uparrow}{0}$=21).

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A NEW SPECIES OF THE GENUS *ALEUROMARGINATUS* CORBETT (ALEYRODIDAE, HEMIPTERA) FROM INDIA

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(*Received 13 April 1956*)

A new aleyrodid (Aleyrodidae, Hemiptera) causing leaf pit-galls on *Bauhinia racemosa* has been named as *Aleuromarginatus bauhiniae* and described in this paper.

In this paper an aleyrodid of the genus *Aleuromarginatus* CORBETT causing pit galls on the undersurface of leaves of *Bauhinia racemosa* is described as a new species. The only species known so far from India is *A. tephrosiae* CORBETT (=*A. indicus* SINGH).

Aleuromarginatus bauhiniae sp. nov.(Figs. 1-3)

Pupal case: Light yellow in colour with a marginal fringe of wax; more or less round but slightly emarginate at thoracic and caudal ends; 1.012 – 1.023 mm long and 0.883 – 0.893 mm wide. Found on the undersurface of leaves in pits showing eruptions on uppersurface.

Margin: Margin strongly toothed with a pore at base of each tooth; 24–27 teeth in 0.1 mm. Thoracic and caudal tracheal pores or combs absent.

Dorsal surface: Subdorsum with light brown shade on all the thoracic segments and up to the middle of the fifth abdominal segment. A row of papillae-like markings evident on subdorsum laterally from the posterior end of cephalic region up to laterad of eighth abdominal segment. Dorsal setae 19 pairs—five pairs on cephalic and five pairs on thoracic regions, 11.5–16.1μ long; nine pairs on abdominal region, 16.1–23.0μ long (a pair on basal abdominal segment, four pairs on abdominal segments from second to fifth, a pair laterad of vasiform orifice (18.5μ long), three pairs sublaterally caudad of vasiform orifice. Longitudinal moulting suture reaches margin of case; transverse moulting suture almost reaches the margin. Abdominal rhachis absent. Dorsum sparsely scattered with pores and porettes. A series of submarginal pores and porettes (about 30 pairs) present Caudal tracheal furrow not indicated. Abdominal segment seven short, pockets distinct.

Vasiform orifice cordate, wider than long (64.4μ × 57.5μ), lateral walls ridged; operculum broadly trapezoidal filling half the orifice (39.1μ × 27.6μ); lingula setose, knobbed. bearing subapically a pair of setae, exposed, included.

Ventral surface: Ventral abdominal setae 23μ long, 62.1μ apart. Antennae slender, long, extending slightly beyond the prothoracic spiracle but reaching base of the mesothoracic leg. All the four pairs of spiracles visible. A minute seta at base of each metathoracic and a pair at the base of each mesothoracic legs evident, marginal setae present: anterior marginal 16.1μ long, posterior marginal 46.0 – 50.6μ long.

Host: *Bauhinia racemosa*

Holotype: Pupal case on slide, *Bauhinia racemosa*, Thirumurthi Hills, 7.6.1975, B. V. DAVID.

Paratypes: Two specimens on a slide deposited in the British Museum, London; 2 specimens on a slide in the United States

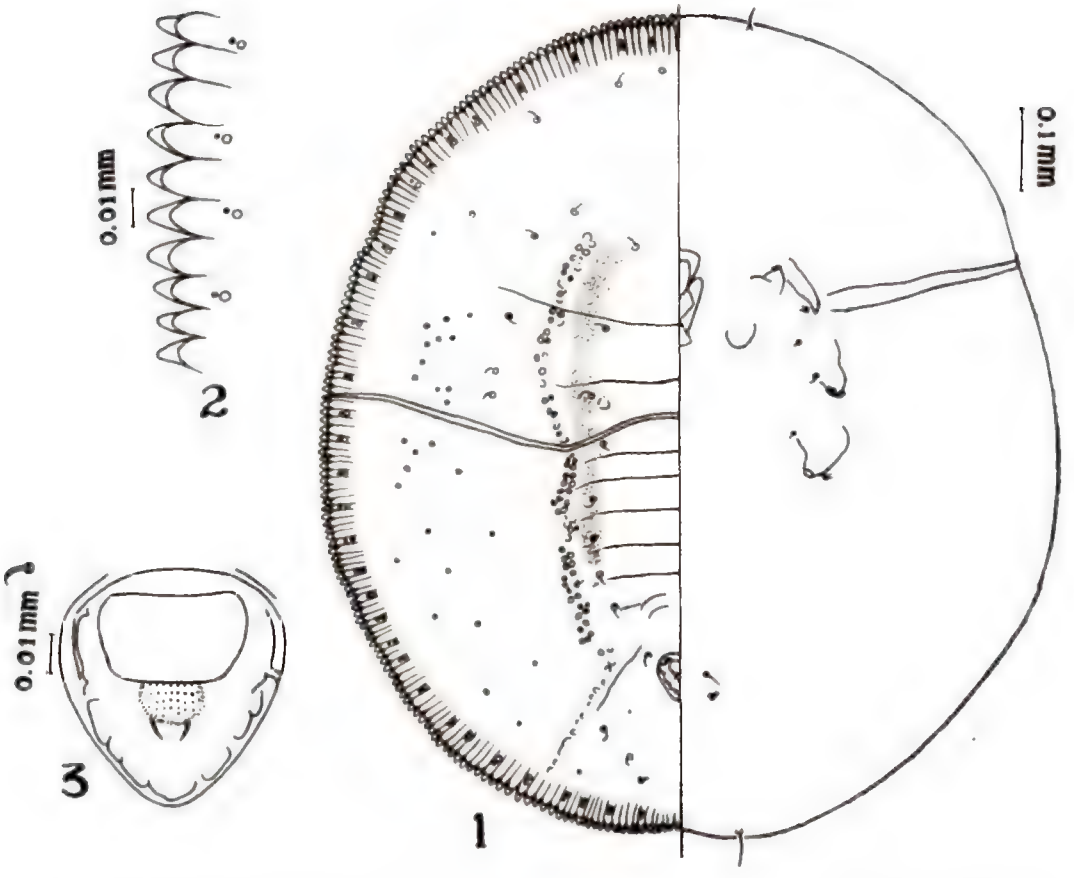

Fig. 1. Pupal case showing dorsal and ventral surfaces. Fig. 2. Margin of case. Fig. 3. Vasiform orifice

Department of Agriculture, Washington; 3 specimens on a slide in the Zoological Survey of India, Calcutta; 2 specimens on a slide in the Division of Entomology, Indian Agricultural Research Institute, New Delhi; and one specimen on a slide and unmounted material with the author.

This species is quite distinct from *A. tephrosiae* CORBETT in being rounded in shape, and dorsum variously shaded with papillae-like markings sublaterally and also in the number and distribution of dorsal setae.

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INVESTIGATIONS ON PARASITES AND PREDATORS OF SOME MAJOR FOREST INSECT PESTS IN INDIA

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Natural enemies of *Adelges* spp., *Pineus laevis* Maskell, *Lymantria obfuscata* Wlk., *Hypsipyla robusta* Moore, *Petrova Cristata*[1] (Walshingham), *Dioryctria* spp. and *Sirex* spp. have been investigated in India with a view to possible biological control of the same or related forest insect pests in various parts of the world. About 50 predatory species attack *Adelges* and some of these have been introduced into Canada and the U.S.A., but without success. Predators of *P. laevis* occurring in India are worth trying against *Pineus pini* (L.) in East Africa and Hawaii. *L. obfuscata* has a large complex of hymenopterous and dipterous parasites, some of which are common to *L. dispar* and occur also in Europe and other areas. A number of *Lymantria* parasites from India are now being bred in the U.S.A. for release against *L. dispar*. *H. robusta* is attacked by over 50 species of parasites and several of these have been shipped to Trinidad and from there to the Windward Islands, British Honduras, Brazil and Mexico, for trial against *Hypsipyla grandella* Zeller. *Trichogrammatoidea robusta* Nagaraja has become established in Trinidad. The pine shoot-borers *P. cristata*[1], *Dioryctria sylvestrella* complex, *D. assamensis* Mutuura, *D. castanea* Bradley and *D. raoi* Mutuura have a large complex of hymenopterous parasites. Many of these have only been identified generically and warrant more detailed studies to evaluate them. *Rhyssa persuasoria himalayensis* Wlk. is a common parasite of siricids. In the light of the work already carried out in India various biocontrol possibilities against forest insect pests are discussed.

INTRODUCTION

The advantages of biological control of forest insect pests have been discussed by various workers (e. g., BALCH, 1958, 1960; PSCHORN-WALCHER, 1961; FRANZ, 1970–71). Several major insect pests of forest trees in India appear suitable targets for biological control and for which the possibilities of introdueing additional natural enemies from other areas are very promising. Attempts to control the teak defoliators *Pyrausta machaeralis* WLK. (Lep., Pyralidae) and *Hyblaea puera* CRAM. (Lep., Hyblaeidae) and the shisham defoliator *Plecoptera reflexa* GN. (Lep., Noctuidae) by transferring some of their indigenous parasites from one area to another within the Indian subcontinent have not been followed up by an assessment of the results achieved (RAO *et al.*, 1971). The first two species are widely distributed in South-east Asia and *Hyblaea* also occurs in parts of Africa (BROWNE, 1968). Parasites from other areas could be introduced into India and *vice versa* to control these pests. With the establishment of its Indian Station in 1957 the Commonwealth Institute of Biological Control has carried out fairly extensive studies on the natural enemies of various other forest pests, *Adelges* spp., *Pineus laevis* MASKELL, *Lymantria obfuscata* WLK., *Hypsipyla robusta* MOORE, *Petrova cristata*[1] (WALSHINGHAM), *Dioryctria* spp. and *Sirex* spp., etc. These species provide not only the basic information to consider additional species for introduction in India from other parts of the world, but also the specific parasites and predators which

[1] Dr. WILLIAM E. MILLER, Principal Insect Ecologist, North Central Forest Experiment Station, U.S.D.A., St. Paul, Minnesota, U.S.A., has recently re-examined the Indian specimens and found that they represent a new species.

warrant trials in other areas for possible biological control of the same or allied forest pests. Many of these natural enemies have been shipped to Canada and the U. S. A., which have largely supported this research work in India.

Balsam woolly aphids

Adelges spp. and *Pineus laevis* MASKELL (Hom., Adelgidae)

Over fifty species of predators are found associated with *Adelges knucheli* S. O. & S. and *Adelges* spp. The most important predator of *A. Knucheli* on *Abies pindrow* in the Western Himalayas is the anthocorid *Tetraphleps abdulghanii* GHAURI. Other important predators in the same region are *Anystis* sp. (Acarina, Anystidae), *Leucopis* spp. (Dipt., Chamaemyiidae), *Chrysopa carnea* STEPHENS, *C. albolineata* KILLINGTON and *Tumeochrysa indica* NEEDHAM (Neur., Chrysopidae), *Hemerobius adelgivorus* KIMMINS (Neur., Hemerobiidae), *Exochomus lituratus* GORHAM and *E. uropygialis* MULS. (Col., Coccinellidae). The Neuroptera feed on various other aphids when *Adelges* is in abeyance but the other predators are restricted to *Adelges* (RAO & GHANI, 1972). In Assam, *Tetraphleps raoi* GHAURI is the most important predator of *Pineus laevis* MASKELL on *Piuns insularis*. Several *Leucopis* spp. also attack *P. laevis* in this area (CHACKO, 1973).

Most of these predators have been shipped to Canada and the U. S. A. and limited field releases made in both countries. None became established. Inability to adapt to a new and different host (*Adelges piceae* (Ratz.)) and unsuitable overwintering conditions are the most probable reasons for their failure (CLARK *et al.*, 1971; AMMAN & SPEERS, 1971).

Lymantria obfuscata WLK. (Lep., Lymantriidae)

The gypsy moth *Lymantria dispar* (L.) does not occur in India, where the closely related *Lymantria obfuscata* WLK. has been confused with it in the past (NAGARAJA *et al.*, 1968). The latter is mainly a pest of *Alnus*, *Populus*, *Salix* and *Quercus*. Five species of egg-parasites, 33 species of larval parasites, 11 species of pupal parasites and 10 spicies of predators of *L. obfuscata* have been recorded in north-western India. Some of the parasites, e.g., *Anastatus bifasciatus* (FONSC.), *Apanteles liparidis* (BOUCHE), *Apanteles porthetriae* MUES., *Brachymeria intermedia*(NEES) and *Compsilura concinnata* MEIG., are also found in Europe and other areas and have already been introduced against the gypsy month in the U. S. A. Rearing techeniques have been developed for two of the Indian parasites, *Rogas indiscretus* REARDON and *Exorista rossica* MESNIL and the chances of their establishment in the U. S. A. are considered to be good (REARDON *et al.*, 1973). Cultures of *R. indiscretus*, *Coccygomimus turionellae* (L.), *Coccygomimus* sp., *Palexorista inconspicua* (MG.) and *P. inconspicuoides* (BAR.) are presently being maintained for field releases (W. W. METTERHOUSE, personal communication). It would also be desirable to try some of the other *Lymantria* parasites against the gypsy month in the U. S. A.

Hypsipyla robusta MOORE (Lep., Pyralidae)

This is a widely distributed major pest of *Cedrela*, *Swietenia* and other Meliaceae in trophical and sub-trophical areas of the Old World. *H. grandella* ZELLER is a serious pest of Meliaceae in the New World. In India *H. robusta* is attacked by 50 species of hymenopterous and dipterous parasites, most of which were found for the first time during surveys carried out by C. I. B. C. The more important of there are *Trichogrammatoidea robusta* NAGARAJA (manuscript species), *Phanerotoma* sp., three *Apanteles* spp., *Afrephialtes latiannulatus* (CAM.), *Tetrastichus spirabilis* WTSTON, *Antrocephalus destructor* WTSTON and *A. renalis* WTSTON. These have been shipped to Trinidad, bred further in the

laboratory and released against *H. grandella* in Trinidad, the Windward Islands and British Honduras (BENNETT & YASEEN, 1972). Some have also been shipped to Brazil and Mexico. In Trinidad *T. robusta* has been recovered several times up to half a mile from the release site.

Petrova cristata (WALSHINGHAM) (Lep., Tortricidae) and **Dioryctria** spp. (Lep., Pyralidae)

Eight species of shoot-borers have been found infesting pines in the Himalayas. These are *Fetrova cristata, Dioryctria sylvestrella* complex, *D. assamensis* MUTUURA and *D. castanea* BRADLEY on *Pinus insularis*, *D. raoi* MUTUURA and two other *Dioryctria* spp. on *Pinus roxburghii*, and *Recurvaria* sp. (Lep., Gelechiidae) on *Pinus longifoial.* Several hymenopterous parasites attack these pine shoot-borers. *P. cristata* is attacked by 15 species, of which *Apanteles* sp. nr. *tachardiae* CAM. is the commonest. Other important ones include a *Campoplex* sp., *Devorgilla* sp., *Bracon* sp. and *Bathystomus* sp. *D. sylvestrella* complex is attacked by 10 parasite species, *D. assamensis* by 8, *D. castanea* by 19 and *D. raoi* by 18 species. *Bathystomus* sp., *Parasierola* sp., *Pimplopterus* sp. nr. *transversus* BRIGG. and *evetriae* UCH., and *Pristomerus* sp. are common to *P. cristata* and to the first three *Dioryctria* spp. *Cremastus* (*Trathala*) sp., *Syzeuctus* sp. and *Trichomma* sp. are major parasites of *D, raoi*, which appear to be effective even at low host densities. *Bathystomus* sp., *Bracon* sp., *Elasmus hyblaeae* FERR., *Parasierola* sp., *Trichomma* sp., *Elachertus nigritulus* ZETT. and *Cremastus* sp. have been successfully bred in the laboratory, using their natural hosts. More detailed studies on the promising species of parasites are required to evalute them as possible candidates for introduction into other areas. In the U. S. A., artificial diets and special techniques have been developed for breeding the European pine shoot-moth *Rhyacionia buoliana* (SCHIFF.) in the laboratory (DATERMAN, 1970). These may prove helpful in breeding the Indian species to build up cultures of their parasites for further studies and for use on biological control programmes.

Sirex spp. and other wood-wasps

In the north-western Himalayas *Sirex cyaneus* F., *S. imperialis* KIRBY, *S. juvencus* L. *Urocerus xanthus* CAM. and *Xeris himalayensis* Bradley are pests of *Abies pindrow* and *Picea morinda. Rhyssa persuasoria himalayensis* WLK. is a common parasite of these Siricids. This has been introduced into Australia and New Zealand but it is not known whether it is estabitshed. On one occasion a specimen of *Megarhyssa* sp. was seen attacking a Siricid larva (possibly *S. imperialis*) (DHARMADHIKARI & ACHAN, 1965).

DISCUSSION

These investigations on some of the major forest insect pests in India have led to the discovery of a complex of parasites and predators, many of which were previously not known, and also provided valuable data on the biology and phenology of the more important species. Only about a dozen of the more than fifty species of predators of *Adelges* ssp. have been tried on a limited scale in Canada and the U. S. A. (CLARK *et al.*, 1971; AMMAN & SPEERS, 1971). Although these failed to become established the prospects of success with some of the other species cannot altogether be ruled out. In both East Africa and Hawaii the Eurasian *Pineus pini* (L.) is reported to be a serious pest of pines. Predators of *Pineus laevis* from India could be introduced into these countries. Various *Lymantria* spp. are highly destructive to forest trees all over the world. In spite of the occurrence of a large

number of parasites *L. obfuscata* sometimes causes extensive defoliation of avenue and orchard trees in the Kashmir valley. Many species of parasites are common to *L. dispar* and *L. obfuscata*. As noted by LEONARD (1974), in certain areas some of the parasites may have established ecological homeostasis with their hosts and it is therefore important to obtain parasites of allied species. There are many more parasites of lymantriids in Asia than Europe and additional species could be introduced not only from Asia into the U. S. A. but also from one part of Asia into another where there is scope for such introduction. According to a recent report, *Hyblaea puera*, *Lymantria flavoneura* JOICEY, *L. ninayi* BETHUNE-BAKER and *Hypsipyla robusta* are important forest pests in Papua New Guina (GRAY & WYLIE, 1974). Parasites of the same or related pests from India may prove useful in controlling these pests. Several species of *Hypsipyla* parasites that exist in India have not been tested against *H. grandella* for want of adequate funds. For the same reason, none of the parasites of pine shoot-borers has yet been tried in other areas. It would thus be seen that based on the work already carried out in this country there are several biocontrol possibilities against forest pests that are well worth exploring fully.

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BOOK REVIEW

PSYLLIDAE OF THE INDIAN SUBCONTINENT by R. N. MATHUR, Indian Council of Agricultural Research, New Delhi. February 1975, xii 429 pp. Rs. 38.50

Psyllids have been a much neglected group of insects in this country as evident from the absence of sufficient concentrated work for the past many years and the publication of this monographic work on the group by the eminent Forest Entomologist, certainly fulfils a long felt need.

The brief introductory section provides a history of work done on this group in India since the first discovery of *Diaphorina guttulata* from Poona in 1890. Information on the methods of collection and rearing and preparation for study is really useful. In the next section on the ecology and economic importance of psyllids, stress is made on the habits, nature of damage and economic importance, along with a detailed host-psyllid list, which in view of the high host specificity of the insects offers valuable data. Mention has also been made of the destructive psyllid species in other countries.

The section on external morphology of adults provides a sound basis for an understanding of the taxonomic treatment given in the next section. The importance of the immature stages in the classification of psyllids has been aptly discussed. A discussion is also presented relating to the relationship of Indian psyllid fauna with those of other zoogeographic areas.

The major section deals with detailed taxonomic studies relating to 101 species belonging to 27 genera and 45 species are described as new to science. Descriptions of the immature stages of 45 species are incorporated and an attempt has been made to establish a correlation between the classification of the adults and the immature stages.

The work is well planned with numerous clear illustrations supporting the descriptions of species and with over 225 references to literature. Besides providing useful information on psyllids, this work will serve as a sound guide to future workers on the group and would undoubtedly remain a work of reference for years to come. The author has to be congratulated on this meritorious work and the Editors, for the fine production.

T. N. ANANTHAKRISHNAN

MYRIAPODA : Symposia of the Zoological Society of London, No. 32 (ed. J. G. BLOWER) Academic Press, London, 1974, 712 pp. £ 14.50

This book comprises forty three papers, most of them original, presented at the Second International Congress of Myriapodology held at the University of Manchester on 5th-12th April 1972, sponsored by the Zoological Society of London and the Centre Internationale de Myriapodologie, Paris. It also includes the Presidential address and a summary of a free discussion on the origin and interrelations of the myriapod groups. At the end is a report of the activities of the International Centre for Myriapodology. The sixty five contributors from various parts of the world cover in this book a wide spectrum of research. If taxonomy comprises the largest single group of papers presented, it is apparently because this aspect has reached fairly advanced stage as far as this group is concerned. Yet the papers indicate the confusion

prevailing in this branch. Among these, one paper attempts to show how scanning electron microscope can elucidate taxonomic problems with ease. There are two excellent papers dealing with segmentation; neuro-endocrinology also finds its proper place. Other papers cover such aspects as reproduction especially ultrastructure of spermatogenesis and ovocyte membranes; development, growth and regeneration. There are papers on various aspects of behaviour such as courtship and mating, food preferences; other aspects of ultra-structure and physiology as well as biochemistry are also represented. The book also includes papers on ecology, population studies, zoogeography and economic zoology as well as toxicology. Each paper is followed by a discussion by participants. These discussions besides being highly informative also serve to throw light on the immense lacunae existing in our knowledge about this group of animals. The various papers in this book represent recent trends in Myriapodology; the book is an index of the present status of this branch of science. Perhaps this is the only comprehensive book on myriapods available in English literature in spite of the fact that twelve papers are either in French or in German. Eventhough the symposium took place in early 1972, the book was published only in 1974; this time lag which was perhaps inevitable appears to have been made good by attempts to update the papers by including references to more recent literature. The book is an asset to Myriapodology; Zoologists in general and research workers in particular will find it extremely valuable. It is sure to stimulate among its readers a renewed interest in myriapods.

V. K. K. PRABHU

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